Vol. III THE JOURNAL OF NUTRITION No. 5

MARCH, 1931

BLOOD STUDIES IN HEMORRHAGIC ANEMIA*

By

H. S. MAYERSON AND HENRY LAURENS

(From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.)

Received for Publication—May 31, 1930

IN THE course of a study of the effects of carbon arc radiation on hemorrhagic anemia, considerable data dealing with the blood cytology of dogs rendered anemic by the method of Whipple and Robscheit-Robbins have accumulated. To the values given by these authors for plasma volume, red cell number and volume and hemoglobin we add values for reticulated cells, total and differential white cells, platelets and red cell fragility, with calculations of corpuscular volume, corpuscular hemoglobin and per cent saturation. The observations here reported were made on 27 short-haired dogs varying in weight from 6 to 18 kilograms. Routine examinations were made in the forenoon. Blood was usually taken from the ear, the first few drops being discarded. Larger quantities when needed were drawn from the femoral vein or artery. Hemoglobin was determined with the Newcomer hemoglobinometer calibrated by oxygen capacity determinations in the Van Slyke and Neill manometric apparatus. Platelets were counted by the direct method of Rees and Ecker; reticulocytes by the method of Haden using brilliant cresyl blue and counterstaining with Wright's stain. Exceptionally fine preparations are obtained by this method. Three hundred red cells are counted, the number of reticulocytes in corresponding fields noted, and the total number computed from the ratio. Cell volume was ascertained in Van Allen hematocrits, using 1.6 per cent Na oxalate and centrifuging for half an hour at 3200 revolutions per minute; plasma volume by the vital red method; specific gravity according to Barbour and Hamilton; fragility by Silvette's method; differential counts by Feemster's method, which makes use of two solutions of Wright's stain in methyl alcohol and ethyl alcohol respectively and buffered water for rinsing and washing the slides. The "corpuscular volume," or average volume of the individual red blood cell, is, as sug-

*Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

gested by Ponder, obtained by dividing the volume of cells per hundred cc. by the number of cells per cu. mm. of blood and is expressed in cubic microns (or 10^{-12} cc.). The "corpuscular Hb," or amount of hemoglobin per cell, is obtained by dividing the hemoglobin per hundred cc. of blood by the number of cells per cu. mm. of blood and is expressed in gms. $\times 10^{-12}$. This calculation, representing a more absolute value, is preferred to that of the color index, since the latter is calculated in terms of per cent hemoglobin which is a variable figure depending on the method and standard used. The per cent saturation, or actual proportion of the substance of each cell taken up by hemoglobin, is obtained by dividing the corpuscular hemoglobin by the corpuscular volume, or directly by dividing the hemoglobin content expressed in gms. per 100 cc. by the hematocrit value expressed in cc. per 100 cc.

Severe secondary anemia was produced according to the procedure outlined by Whipple and Robscheit-Robbins. All of the animals were obtained from the pound. The criticism of the use of pound dogs does not appeal to us, for it is possible by not unreasonably careful selection and preparation to obtain suitable pound animals giving quite constant results. The animals, when received, are placed on the standard diet and are always kept for weeks in the kennels in order that they may become thoroughly accustomed to their surroundings. They are then bled, 3, 4 or more times at intervals of 1 or 2 days to one-third or one-half of their original hemoglobin level and this level then maintained by blood withdrawals graded as to their frequency and amount by ascertained hemoglobin and hematocrit values, due consideration being given to fluctuations in plasma volume. Hemoglobin was determined in samples of the blood thus withdrawn, thereby giving the information as to how much hemoglobin was produced over and above the presumably fairly constant maintenance factor. Some animals possess a considerable reserve of hemoglobin-forming materials which makes it necessary to follow them for protracted periods because, until this reserve is exhausted, an anemic level cannot be maintained.

Bread S, containing salmon, (Whipple and Robscheit-Robbins, 1925a) was used in all the experiments. This bread is a complete diet for an adult dog and will apparently maintain an animal in health indefinitely. As Whipple and his collaborators have emphasized, the ration keeps the hemoglobin production at a low level, the average weekly production in 15 of our experiments being 4 gms. There are, of course, individual differences and some dogs show consistently high values. Thus the average amount of hemoglobin removed weekly from Dog H during a period of 11 weeks was

9 gms. as compared with the weekly average of 1 gm. for Dog J over a 6-weeks' period.

Dried peaches were added to the diet in 3 experiments, dried apricots in 3, lettuce in 5, and some animals received each of these additional foodstuffs at different periods of the experiments. These substances are only mildly favorable for hemoglobin production (Robscheit-Robbins and Whipple, 1927a and Robscheit-Robbins, 1929). In observations here reported the average weekly hemoglobin production when apricots were fed was 15 gms., when lettuce, 10 gms., and when peaches, 7 gms. There are, of course, individual differences, some animals giving better responses to lettuce than to apricots, others showing better regeneration with peaches than with lettuce. The relatively poor reaction to peaches may have been due to the greater difficulty in getting the animals to consume them. One animal, Dog U, proved refractory to all three substances, showing no increase in hemoglobin production for a period of 12 weeks.

The efficiency of this diet in maintaining hemoglobin at an anemic level and in providing a few grams of hemoglobin above the unknown maintenance factor is in contrast to that of the Cowgill diet (1923). This diet, made up of casein, butter fat, lard, sugar and a salt mixture, was used as a maintenance diet. When this diet exclusively is fed, the hemoglobin level of normal animals remains relatively constant over long periods of time (see Drabkin and Waggoner, 1929), but not so in animals made anemic by hemorrhage. To check its efficiency with the salmon bread diet, it was fed to three animals. One of these animals, Dog W, had been anemic and on the bread S diet for over 2 months, during which time the efficiency of lettuce and apricots had been demonstrated, and the dog continued to show the usual responses to these materials. When placed on the Cowgill diet, the hemoglobin level began to decrease almost immediately from a value of 6.5 gms. per cent to 4.25 gms. per cent over a period of 6 weeks, despite the fact that only about 5 gms. of hemoglobin, representing the amount of blood needed for routine examinations, had been removed over the entire period. On reverting to the bread S diet, the hemoglobin level began to rise slowly. The experience with the other two dogs was similar.

Whipple and Robscheit-Robbins tabulate the changes in color index and in hemoglobin index (hemoglobin per cent divided by hematocrit per cent) under various conditions and discuss their significance. We have calculated the volume, hemoglobin content and per cent saturation of the individual cell. The individual cell volume expressed in cubic microns (10^{-12} cc.) is the corpuscular volume, while the hemoglobin content in

10^{-12} grams is the corpuscular hemoglobin. The calculation of the corpuscular hemoglobin and per cent saturation instead of the color and hemoglobin indices is preferable, we believe, since it involves the statement of hemoglobin content in grams rather than in per cent, the absolute value of which varies with the particular method and standard used. An analysis of the changes in corpuscular volume and corpuscular hemoglobin shows that if an hemorrhagic animal is kept on the bread S diet exclusively for any length of time the corpuscular volume and corpuscular hemoglobin gradually decrease, as has been shown to be the case in clinical secondary anemia. Table I shows a typical case. The figures in the table represent the values at the end of any given week. Likewise the figure for hemoglobin removed represents the total amount of hemoglobin removed during that week. The hemoglobin figures noted for the first week of bread S feeding represent the production of hemoglobin due to 7 days of bread feeding. After this animal had been placed on the bread S diet, a three-week's preliminary period was necessary to diminish its storage of reserve factors and to reach a fairly steady anemic level. Despite this procedure, this animal was consistently high in hemoglobin production. The values listed in the table begin with those obtained after this period and comprise eleven weeks during which the bread S was fed with no addition, the hemo-

TABLE I

DOG H, COLLIE, FEMALE, ADULT

FED 400 GMS. BREAD S PLUS 50 GMS. SALMON DAILY

Weeks	Weight K	Plasma vol. c.c.	Spec. grav.	R.B.C. millions	Retic. p.c. of R.B.C.	R.B.C. hemat. %	Corp. vol. cu. μ	Hb level gms., %
1	16.0	913.4	1.0372	3.83	3.0	18.5	49.5	6.22
2	16.0		1.0375	4.26	3.0	21.0	49.2	6.76
3	15.9	853.3	1.0356	4.34	3.0	20.0	46.0	6.09
4	15.9	981.4	1.0335	5.20	4.0	24.0	46.1	7.44
5	15.9	742.0	1.0352	4.94	4.0	21.0	42.5	6.09
6	15.8	711.0	1.0388	6.90	16.7	25.0	36.2	6.76
7	15.9	603.6	1.0364	5.54	14.0	23.0	41.5	6.58
8	16.0		1.0340	5.46		18.0	33.2	5.92
9	15.8		1.0371	6.42	10.0	20.0	31.2	6.42
10	15.6		1.0349	5.80	10.0	20.0	34.4	5.42
11	15.6	754.0	1.0351	6.64	11.2	21.0	31.6	6.59

$$\text{Corpuscular Volume in cu. } \mu = \frac{\text{Vol. (c.c. per 100 c.c.)}}{\text{R.B.C. (in millions)}}$$

$$\text{Corpuscular Hb in } 10^{-12} \text{ gms.} = \frac{\text{Hb (gms. per 100 c.c.)}}{\text{R.B.C. (in millions)}}$$

globin level being kept constant by periodic bleedings. As the experimental period progresses, there is a gradual increase in the red cell number so that at the end of eleven weeks the count is almost double that of the first week. There is, however, relatively little change in the hematocrit values, signifying the production of cells of smaller volume. Along with the decrease in corpuscular volume, there is a parallel but somewhat greater decrease in corpuscular hemoglobin to a value approximately 60 per cent of the original anemic level at the beginning of the experiment. Since the total amount of hemoglobin is more or less constant, the percentage of hemoglobin in each smaller cell remains relatively unchanged. The stability of this factor is discussed in another paper (Mayerson, 1930) and attention directed to the surprisingly narrow distribution of the average values of sixty animals. The average per cent saturation for the eleven weeks of this experiment was 30.6 per cent, which is within the normal range and close to the average figure of 34.3 per cent.

In most of their experiments Robscheit-Robbins and Whipple (1927b) find that the feeding of substances which are more or less potent in hemoglobin regeneration leads to an increase in hemoglobin index, that is, a more abundant production of hemoglobin than of stroma. Thus the feeding of beef and pig kidney, gizzard, liver, etc., produces a definite increase in the hemoglobin and color indices while with the feeding of fish

TABLE I (Continued)

DOG H, COLLIE, FEMALE, ADULT

FED 400 GMS. BREAD S PLUS 50 GMS. SALMON DAILY

Hb removed gms.	Corp. Hb gms. X 10^{-11}	Saturation %	Whites	Differential			Platelets thousands
				Lymphocytes	Neutrophiles	Eosinophiles	
10.67	16.2	32.7	10200	30	69	1	310
16.48	15.8	32.1	7600				
7.00	14.0	30.4	8200	35	64	1	290
8.12	14.3	31.0	8800				
8.79	12.3	28.9	7000	33	66	1	310
8.78	9.8	27.0	9000				
10.14	11.6	27.9	9000	34	66	0	220
8.60	10.9	32.8	7500				
11.10	10.0	32.1	9400				
8.35	10.0	29.6	8000	37	63	0	280
8.60	9.9	31.6	12000	28	72	0	540

$$\text{Saturation in p.c.} = \frac{\text{Hb(gms. per 100 c.c.)}}{\text{Vol. (c.c. per 100 c.c.)}}$$

liver there is no decided change. Cream, milk and butter (Whipple and Robscheit-Robbins, 1927), however, all show color and hemoglobin indices which tend to fall below the control period. This is especially true in experiments where the substances fed were decidedly inert (Whipple and Robscheit-Robbins, 1925b; Elden, Sperry, Robscheit-Robbins and Whipple, 1928) as in Table No. 131 for Dog 25–23 taken from the latter paper (page 579) here reproduced as Table II, the corpuscular volume and corpuscular hemoglobin having been calculated from their data for compari-

TABLE II*
DOG 25–23 BULL, MALE, ADULT

Diet Periods 1 Week Each. Food: grams per day	R.B.C. millions	R.B.C. hemato-crit %	Hb level gms. %	Corp. Vol. cu. μ	Corp. Hb gms. X 10^{-12}	Satura-tion %
Bread 250, Salmon 100, Klim 25	5.2	22.7	6.07	43.6	11.6	26.5
Copper Sulphate 19	5.3	23.1	5.91	43.5	11.7	26.8
Copper Sulphate 19	5.9	19.5	5.10	33.0	8.7	26.2
Copper Sulphate 19	5.3	24.4	6.07	46.0	11.4	24.7
Bread 300, Salmon 100, Klim 25	5.3	23.4	5.79	44.1	10.9	24.7
Bread 300, Salmon 100, Klim 25	6.4	25.9	6.34	40.4	9.9	24.5
Bread 300, Salmon 100, Klim 25	6.7	21.6	5.65	32.2	8.4	26.2
Bread 350, Salmon 100, Klim 25	6.5	26.4	6.62	40.6	10.1	24.8
Copper Sulphate 252	6.4	24.8	6.90	39.0	10.7	27.4
Copper Sulphate 252	6.9	25.6	6.90	37.1	10.0	26.9
Bread 350, Salmon 75, Klim 25	7.2	26.0	6.34	36.1	8.8	24.4
Bread 350, Salmon 75, Klim 25	8.0	21.9	6.07	30.0	7.6	25.3
Bread 400, Salmon 75, Klim 25	7.0	21.5	5.91	30.7	8.4	27.4
Bread 400, Salmon 75, Klim 25	6.6	20.8	5.79	31.5	8.8	27.5
Bread 400, Salmon 75, Klim 25	6.0	21.4	5.65	35.6	8.9	25.0

* Being Table #131 of Elden, Sperry, Robscheit-Robbins and Whipple (1928) to which have been added calculations of corpuscular volume, corpuscular hemoglobin and per cent saturation.

son with Table I. In the interpretation of their results these authors suggest that the low color and hemoglobin indices may indicate that the materials

for the construction of stroma are more abundant in these diets than are the construction materials for hemoglobin production. In the light of the results here reported it seems more likely that the changes are but the expression of a long continued drain on the organism fed on foodstuffs relatively inert for hemoglobin production. The changes are similar to those taking place in the adjustment to the stress of long continued O_2 want, which results in the manufacture of small, pale cells.

Similar changes were reported following carbon arc irradiation of normal animals (Mayerson and Laurens, 1928), small pale cells being formed in the post-irradiation periods. The suggested explanation of these changes was that there was a minimum formation of new cells during the irradiation period, the system calling chiefly on its reserves, while in the post-irradiation period the bone marrow having exhausted its store is making new cells, poor in hemoglobin and of a low color index. In the present experiments it may be assumed that the decreased size of the cells is due, not only to the removal of cells of normal size by the hemorrhage (Price-Jones, 1922) but also, to the unfavorable conditions of O_2 want due to the absence of any increase in hemoglobin production. Mills (1925) and Emmons (1927) have brought forth evidence indicating that hemoglobin is superficially distributed in the cell, so that, as Whipple and Robscheit-Robbins point out, these small cells are probably more efficient in O_2 exchange and transfer because of the relative increase in cell surface per unit mass. That this is the probable explanation of the changes observed in these experiments is strengthened by their absence under conditions which are mildly favorable to hemoglobin production. An increase in the number of red cells under these conditions usually results in a concomitant increase in the hematocrit values and in the hemoglobin. Thus in the eleven experiments where dried peaches, apricots or lettuce were added to the diet, in six there was little or no change in corpuscular volume; in three an actual increase, and in only two was there a slight decrease during periods of from two to four weeks. The same is true of the corpuscular hemoglobin which shows a decrease in only these two experiments. Table III shows the effect of adding apricots to the standard diet. Table IV is Robscheit-Robbins' and Whipple's (1927a) Table No. 96, with the addition of corpuscular volume and corpuscular hemoglobin as calculated from their data. This table is typical of many of their results as well as of ours. With the addition of substances which may be only mildly favorable for regeneration, there is an absence of the changes previously observed in stroma formation, cell volume and corpuscular hemoglobin. An

TABLE III

DOG R, TERRIER, ADULT, FEMALE

FED 300 GMS. BREAD S PLUS 200 GMS. APRICOTS

Weeks	Weight K	Plasma vol. c.c.	Spec. grav.	R.B.C. millions	Retic. p.c. of R.B.C.	R.B.C. hemat. %	Corp. vol. cu. μ	Hb level gms., %
1	7.8	610		5.54	4.7	22.5	40.6	6.66
2			1.0380	5.06	7.3	28.0	55.3	7.11
3	7.9	565	1.0391	4.96	10.0	28.0	56.3	7.04
4			1.0434	5.25	5.3	33.5	63.9	8.86
5	7.8	545	1.0385	5.36	4.7	25.0	46.6	7.79
6			1.0357	4.63	6.7	22.5	48.5	6.50
7	7.8	601	1.0343	4.64	4.0	27.0	61.3	8.22
8	7.7		1.0407	4.60	7.0	26.5	57.6	7.80

attractive view in regard to this is that these substances reduce the strain on the bone marrow by providing it with hemoglobin-forming substance. Scott (1923), working on hemorrhagic anemia in rats fed on a milk diet, reports similar findings. The anemia existing under these conditions persists for two to three weeks after the last bleeding and is accompanied by a low color index. If green stuff is added to the diet, this fall in the color index is absent.

The course of the reticulated cells varies in different animals. The average percentage is usually 3, when the anemia level is established. This value is maintained with minor fluctuations in many of the experiments, while in other cases temporary increases frequently occur. Dog H for example (Table I), showed fluctuations of from 1 to 4 per cent for the first five weeks of the experimental period. The count then rose to 16.5 per cent, dropping to 14 per cent at the end of the tenth week. This increase in reticulated cells is part and parcel of the increase in total red cell count which has previously been mentioned. It begins at a time when the hemoglobin level is low and no large quantity of blood is being removed. It seems plausible to explain the change as a response of the bone marrow to the stimulus of oxygen deficiency due to the diminished hemoglobin. Robertson and Bock (1919) make a similar interpretation of their findings of marked reticulocytosis following severe hemorrhage.

Since the experimental method was concerned with maintaining a steady hemoglobin level, the repeated bleeding served as an additional stimulus to keep the number of reticulocytes high. In other experiments

TABLE III (Continued)
DOG R, TERRIER, ADULT, FEMALE
FED 300 GMS. BREAD S PLUS 200 GMS. APRICOTS

Hb removed gms.	Corp. Hb gms. X 10^{-13}	Saturation %	Whites	Differential			Platelets thousands
				Lymphocytes	Neutrophiles	Eosinophiles	
	12.0	29.5	9360	39	61	0	770
7.41	13.8	24.9	6075				
	14.0	24.8	16100	30	70	0	830
10.19	16.8	26.2	11775	27	64	0	620
5.82	14.5	31.1	16050	25	75	0	850
13.35	14.0	28.8	7850	26	74	0	1550
6.58	18.6	30.3	11800	26	74	0	720
7.02	16.9	29.3	13425	20	79	1	2800

the relatively large increases in reticulocytes are accompanied by only slight and temporary increases in red cell number and quickly subside. The periodic hemorrhages are, as would be expected, followed by temporary showers of reticulated cells. On the other hand, some animals maintain levels of 6 and 8 per cent reticulated cells and yet show no hemoglobin regeneration over and above the anemic level. When peaches, apricots, or lettuce are added to the bread S diet, the reticulocyte percentage becomes lower on the whole than when such additions are not made (Table III). Riecker (1928) reported little or no change in the reticulocyte percentages of dogs in which severe anemia had been produced by the method of Whipple and Robscheit-Robbins and fed bread C. He concluded that since any stress on the bone marrow would have been indicated by more pronounced reticulocyte increases, the method was testing the power of hemoglobin regeneration only and not the mechanism of cell production in the bone marrow. An analysis of his protocols shows that the actual control anemic periods were relatively short. Only one animal (Dog 72) was fed bread C alone for any considerable period (five weeks) after the anemic period had been reached. In this animal the reticulocyte percentage increased from 1 to about 4 per cent, while the anemia was being produced, and fluctuated by about 7 per cent for the last two weeks previous to the addition of Blaud's pills, during the administration of which the percentage began to decrease.

The resistance of the erythrocytes to sodium oxalate solutions was determined in seventeen experiments. The results were somewhat inconsist-

TABLE IV
DOG 24–22 COACH, FEMALE, YOUNG ADULT

Diet Periods 1 Week Each. Food: grams per day	R.B.C. millions	R.B.C. hemato-crit %	Hb level gms. p.c.	Corp. Vol. cu. μ	Corp. Hb gms. X 10^{-12}	Satura-tion %
Raisins 300, bread 350	6.0	27.0	7.28	45.0	12.1	26.9
Raisins 250, bread 350	6.2	27.4	6.76	44.2	10.9	24.6
Bread 450, beef 75	5.0	22.4	6.07	44.8	12.1	27.0
Bread 450, beef 75	5.3	23.2	5.93	43.8	11.2	27.8
Dried peaches 300, bread 350	5.8	25.5	6.90	44.0	11.9	27.5
Dried peaches 300, bread 350	5.6	24.5	6.35	43.8	11.3	25.8
Bread 450, salmon 50	4.6	22.7	5.80	47.2	12.6	26.7
Bread 450, salmon 50	5.2	24.7	6.62	47.5	12.7	26.7

* Being part of Table #96 of Robscheit-Robbins and Whipple (1927a) to which have been added calculations of corpuscular volume, corpuscular hemoglobin and per cent saturation.

ent and bear no relation to the diet fed. In six experiments there was little or no change during the anemic period, even for periods of ten and eleven weeks. In a similar number of experiments the resistance was increased. In the remaining cases the resistance was slightly decreased. Kesten and Zucker (1929) recently reported that hemolysis due to saponin is somewhat slower, but not markedly so in secondary anemia due to repeated hemorrhages from duodenal and gastric ulcers.

The platelets show no marked changes, except for occasional showers which occur more frequently when additions are made to the bread diet. These showers are sometimes striking (see Table III). The average platelet count of Dog W, fed 400 gms. bread and 200 gms. apricots daily, was 775,000 for the first week. At the end of the second week, the count spurted to 2,080,000, receded to 860,000 and rose to and remained at about three million the following week. Similar changes occurred in most of the experiments in which peaches, apricots or lettuce were added to the diet. The total white and differential counts do not show any marked or consistent changes except in two experiments. Dog N, fed 400 gms. of bread S for a period of ten weeks, showed a gradual decrease in lymphocytes beginning with the second week of the anemia period. At the end of this time the count showed only 9 per cent lymphocytes (pre-hemorrhagic level 21 per cent), the neutrophiles being correspondingly increased to 90 per cent. Dog T, fed 300 gms. of bread plus 150 gms. of peaches, showed similar changes.

SUMMARY

A study is presented of the blood cytology in severe secondary anemia produced and maintained for varying lengths of time in twenty-seven dogs following the method of Whipple and Robscheit-Robbins. In dogs fed the standard bread S there is an average weekly production of 4 gms. over and above the maintenance level. When apricots are added this becomes 15 gms., when lettuce 10 gms. and when peaches 7 gms. Cowgill's diet was found to be inadequate for maintaining a constant hemoglobin level under similar conditions.

After several weeks of bread S feeding, there is a gradual increase in red cell number accompanied by a decrease in the corpuscular volume and corpuscular hemoglobin. The percentage of hemoglobin in these smaller cells, however, remains relatively unchanged. If lettuce, apricots or peaches are added to the diet, these changes to do not occur and they are interpreted as being compensatory reactions to the oxygen deficiency occasioned by the low hemoglobin when the severe anemic level is maintained.

BIBLIOGRAPHY

Cowgill, G. R., 1923, *Jour. Biol. Chem.*, LVI, 725.
Drabkin, D. L., and C. S. Waggoner, 1929, *Science*, LXIX, 480.
Elden, C. A., W. M. Sperry, F. S. Robscheit-Robbins, and G. H. Whipple, 1928, *Jour. Biol. Chem.*, LXXIX, 577.
Emmons, W. F., 1927, *Jour. Physiol.*, LXIV, 215.
Haden, R. L., 1923, *Arch. Int. Med.*, XXXI, 766.
Kesten, H. D., and T. F. Zucker, 1929, *Amer. Jour. Physiol.*, LXXXVII, 274.
Mayerson, H. S., 1930, *Anat. Rec.* XLVII, 239.
Mayerson, H. S., and H. Laurens, 1928, *Amer. Jour. Physiol.*, LXXXVI, 1.
McCay, C. M., 1928, *Ibid.*, LXXXIV, 16.
Mills, E. S., 1925, *Arch. Int. Med.*, XXXV, 760.
Price-Jones, C., 1922, *Jour. Path. and Bact.*, XXV, 487.
Riecker, H., 1928, *Jour. Clin. Invest.*, V, 141.
Robscheit-Robbins, F. S., 1929, *Physiol. Rev.*, IX, 666.
Robscheit-Robbins, F. S., and G. H. Whipple, 1927a, *Amer. Jour. Physiol.*, LXXX, 400. 1927b, *Ibid.*, LXXIX, 271.
Robertson, O. H., and A. V. Bock, 1919, *Jour. Exper. Med.* XXXIX, 139.
Scott, J. M. O., 1923, *Biochem. Jour.*, XVII, 166.
Whipple, G. H., and F. S. Robscheit-Robbins, 1925a, *Amer. Jour. Physiol.* LXXII, 395. 1925b, *Ibid.*, LXXII, 419. 1927, *Ibid.*, LXXIX, 280.

THE EFFECTS OF RADIANT ENERGY ON HEMORRHAGIC ANEMIA*

By

HENRY LAURENS AND H. S. MAYERSON

(From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.)

Received for Publication—May 31, 1930

PREVIOUS communications (Miles and Laurens, 1926; Mayerson, 1927; Mayerson and Laurens, 1928) have indicated a stimulation of the hematopoietic system in normal dogs by carbon arc radiation. Repeated massive exposures increased the red cell number from 10 to 19 per cent, the increases being maintained for from 3 to 6 weeks after the last irradiation. The suggestion was made at the time that, since radiation acts as a hematopoietic stimulus to the normal, relatively stable organism, it seemed plausible to expect it to be particularly efficient in effecting regeneration in anemic conditions.

The literature dealing with the effects of radiant energy on the hematopoietic system has been reviewed by Laurens (1928) and by Robscheit-Robbins (1929). Their summaries indicate a lack of agreement in the results obtained by different investigators without question due to the presence of variables, such as environment, diet, unstandardized radiation sources, etc. This is especially true of many clinical studies in which favorable results have been ascribed to radiation which might just as well have been due to the improved dietary and hygienic regimen during the investigation. Conclusions based on red cell counts or hemoglobin determinations alone can hardly be held reliable unless these determinations are correlated with changes in the other characters of the blood, such as cell and plasma volume, reticulocytes, etc. The use of unstandardized sources and the failure to give energy specifications render an exact repetition of observations and a comparison of results impossible.

The observations, the results of which are here reported, have to do with the effects of radiation on hemorrhagic anemia in dogs. Although, as Mitchell (1926) points out, this method may possibly fall short of simulating and of solving the problem of the naturally occurring anemias in man, it does afford a convenient method of obtaining accurate, comparative results in experimental animals. In later contributions the effects of radiation on various types of experimentally produced hemolytic anemias will be reported.

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

The methods of estimating the blood constituents and the calculations used have been outlined in the preceding paper. The chief source of radiation was the "Pan Ray" flaming carbon arc burning "sunshine" carbons. Operating at 24 to 28 A and 55 to 60 V, the energy emitted has an average value of 0.820 gm. cal. per sq. cm. per minute incident at 1 M. and has a distribution of 5 to 6 per cent in the ultraviolet, 29 to 31 per cent in the luminous and 63 to 66 per cent in the infra red as determined by dispersing the energy and ascertaining the amount in small bands throughout the spectrum as well as by the use of screens which split the spectrum into the three major divisions. A Cooper-Hewitt air-cooled quartz mercury-vapor arc was used in some of the experiments. This source, operating at 4 to 5 A and 70 to 80 V, emits an average total energy of 0.0619 g.m. cal. per sq. cm. per minute incident at 1 M. When the energy of the quartz mercury vapor lamp is dispersed, its distribution is as follows: 48 per cent in the ultraviolet, 37 per cent in the luminous (390–710 mμ), and 15 per cent in the infra red. When the distribution is determined by means of screens it is: 13 per cent ultraviolet, 7 per cent luminous (400–1400 mμ) and 80 per cent infra red. The smaller percentage of infra red when the energy distribution is determined by the dispersion method is due to the fact that this energy, coming from the glowing tungsten anode and the metal parts of the lamp, does not to any extent exert its effect on the thermopile because the slit of the spectrometer is centered on the middle of the quartz mercury container. The infra red energy actually emitted by the quartz mercury vapor arc itself is negligible. But the tungsten anode of the Cooper-Hewitt model, as well as the quartz container and the hood, emits a long waved infra red and this shows up in the screen method of determining the distribution though not in the dispersion method. The methods used in measuring and specifying the energy have already been described (Laurens and Mayerson, 1929).

The first two experiments were designed to test the effects of carbon-arc radiation on simple anemia produced by bleeding dogs to one-half or one-third their blood volume. These animals were fed a standard casein diet (Cowgill, 1923) and the requisite amount of blood was drawn by two or three successive bleedings. The abdominal area of the animals was then irradiated and determinations continued until the pre-hemorrhagic levels were recovered. In the first experiment the animal was given 10 one-half hour exposures at a meter's distance (24 g. cal. per sq. cm. each) on alternate days, while the second animal was subjected to 9 such exposures.

The response of the erythrocytes in each irradiated animal was somewhat quicker and greater than that of the controls, recovery being practi-

cally complete in 22 days in the first, and 13 days in the second, as compared with 26 and 27 days in the control animals. The changes in hemoglobin roughly paralleled those in the reds. The corpuscular hemoglobin fluctuated about the normal values with no distinction between the irradiated and non-irradiated animals. The hematocrit showed a much slower recovery in the irradiated animals than in the controls and, while the corpuscular volumes of the irradiated animals were high for about a week after hemorrhage, they decreased gradually to values below the original in the latter part of the irradiation and post-irradiation periods. The per cent saturation of the red cells of both animals was normal. Small cells of approximately normal saturation were observed in previous work on the irradiation of normal animals. The specific gravity, in general, followed the course of the reds, and there was no difference in the fragility of the red cells. Whites and platelets showed no significant changes.

Osato and Tanaka (1929) reported experiments on dogs irradiated weekly with a Bach mercury vapor lamp. The regeneration of blood, as measured by red cell and hemoglobin determinations, was much more active in irradiated, splenectomized animals than in non-irradiated splenectomized animals. Their results on irradiating non-splenectomized animals are hardly as striking, since both the irradiated and non-irradiated animals recover to pre-hemorrhagic levels in about the same time. The blood iron is increased at the expense of the reserve iron in the liver and spleen, or in the liver alone in splenectomized animals.

The rapidity of hematopoietic regeneration in the dog and its extreme variability (14–28 days) in different animals has been pointed out by various observers. Different animals vary so markedly in the amount of reserve material which they possess, that it is difficult to interpret results obtained unless these reserves are first depleted. To avoid these difficulties the experimental method was changed, and in the succeeding experiments a severe secondary anemia was produced according to the procedure outlined by Whipple and Robscheit-Robbins, with the modification that radiation is substituted for test food substances. The method is described in detail in the preceding paper and the same animals there referred to were used in this study. In each case the abdomen and thorax were irradiated. The animals were followed for periods of at least two weeks before and two weeks after the irradiation period. Control animals were followed simultaneously throughout the periods of observation, being subjected to the same procedure except for the irradiation.

Previous work showed that massive doses were most efficacious in affecting the hematopoietic system in normal animals and eight dogs were

thus treated, seven with the carbon arc, and one with the mercury arc. Two dogs were irradiated once at 60 cm. for 45 and 60 minutes respectively (100 and 133 g. cal. per sq. cm.); two animals were irradiated 3 and 8 times respectively on alternate days for 60 minutes at 80 cm. (76 g. cal. per sq. cm.); and a fifth animal was irradiated for 1 hour at 60 cm. (133 g. cal. per sq. cm.) followed the next day by a 30-minute exposure at the same distance. Two weeks later it was again irradiated for 60 minutes at 80 cm. and the same exposure repeated twice at intervals of a week. The sixth animal was irradiated for 90 minutes at 60 cm. (200 g. cal. per sq. cm.) followed in two weeks by 60 minutes at 80 cm. and again by a similar exposure a week later. The seventh animal was irradiated twice for 60 minutes at 60 cm. with a 3-week interval between exposures while the eighth animal was irradiated once for 45 minutes at 60 cm. (8.37 g. cal. per sq. cm.) with the quartz mercury vapor lamp.

The most pronounced effects are on the number of erythrocytes and reticulocytes, with no corresponding changes in hemoglobin formation. Irradiation is followed by a greater or lesser increase in the number of red cells. The rise usually begins immediately or within a few days after the first exposure and continues after the irradiation is stopped but there seems to be no constant relationship between the increase and the length of exposures or the intervals between them. There is little difference between the results of irradiation with the carbon arc and with the quartz mercury vapor lamp. In the two experiments where massive carbon arc irradiations were given every other day, the maximum red count did not occur until the irradiation was discontinued, while in the others it was present at the end of the first week of the irradiation period. Figure 1 shows a typical case. This animal was observed for a month after anemic levels had been established, during which period the various constituents showed only minor fluctuations. During the next two weeks the dog was irradiated with the carbon arc for 60 minutes at 80 cm. every other day for a total of 8 times. The number of reds began to rise after the fourth irradiation and continued to do so even after the exposures were discontinued so that at the end of the ninth week (3 weeks after the last irradiation) the count was 7.54 millions, which is to be compared with the pre-irradiation level of approximately 4 millions and the pre-hemorrhagic value of 8.35 millions. This high level was maintained for about a week. The count at the end of the thirteenth week (7 weeks after the last exposure) was still about a million and a half above the control pre-irradiation level of 4 millions. This rise in reds was accompanied by a parallel increase in reticulocytes to a value three times normal at the end of the tenth week, the level being

still high seven weeks after the last irradiation. These changes are similar to those reported in the preceding paper dealing with the effects of hemorrhage alone but are of much greater duration and magnitude. It is clear that they were due to the irradiation and were not the result of the long-continued diet because the values decreased in the latter part of the post-irradiation period.

In all but two experiments (following single carbon arc exposures of 1 hour at 60 cm. and 45 minutes at 60 cm.) the percentage of reticulocytes markedly increased (at least double). The increase usually preceded that in the reds, beginning immediately after the first irradiation and gradually increasing to a maximum after the third or fourth exposure and remaining at this level. In some cases there was a further, and sometimes more marked, secondary rise during the first or second week of the post-irradiation period. In two experiments the values were still high 6 and 7 weeks respectively after the last irradiation. This reticulocyte increase is not due to an increased amount of bleeding, for in all cases there was very little hemoglobin production and only the minimum amount of blood necessary for the determinations was being removed. In one experiment the reticulocytes were at a higher level during the irradiation period than they were during the pre-irradiation period when considerable amounts of blood were being withdrawn. These increases in red cell number and reticulocytes were not accompanied by corresponding changes in hematocrit values, which remained low, and thus the corpuscular volume diminished as the number of cells increased. All of these changes are graphically shown in Figure 1.

In only one of these experiments (discussed in detail below) was there any indication of increased hemoglobin formation as a result of the irradiation. In the two experiments, where the animals were irradiated every other day for two weeks, the hemoglobin formation stops immediately on commencement of the irradiation and does not resume until 2 and 4 weeks respectively after the irradiations are over (See the figure). In the other experiments this inhibition of hemoglobin production is not so marked, but the amount formed during and after the irradiation period is several grams less than in the pre-irradiation period. This lack of increased hemoglobin production along with the rise in the number of red cells results in a gradual decrease in corpuscular hemoglobin. Spence and Griffith (1927) also found that cell production proceeded more rapidly than did hemoglobin formation after carbon and mercury arc irradiation. Since the decrease in corpuscular hemoglobin runs almost parallel to that in the corpuscular volume, the per cent saturation of these cells remains very nearly

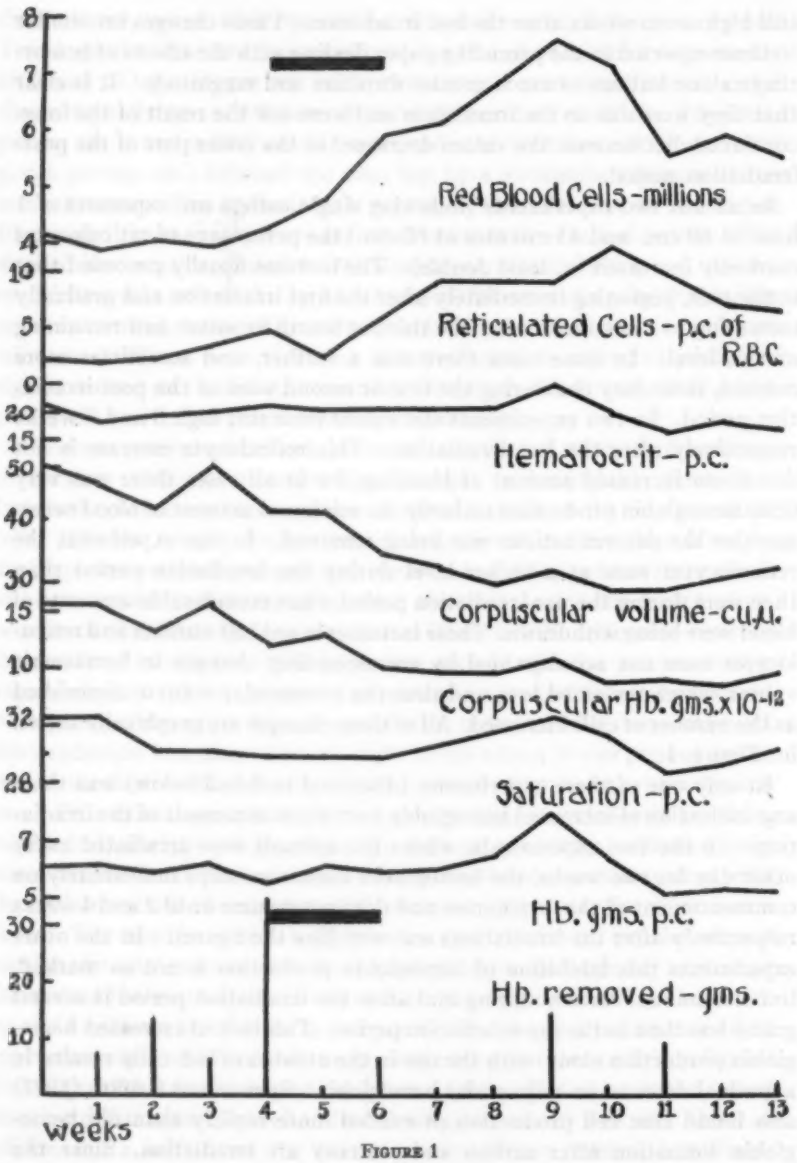
Red Blood Cells - millions
Reticulated Cells - p.c. of R.B.C.
Hematocrit - p.c.
Corpuscular volume - cu.μ.
Corpuscular Hb. gms. x 10⁻¹²
Saturation - p.c.
Hb. gms. p.c.
Hb. removed - gms.
weeks
1 2 3 4 5 6 7 8 9 10 11 12 13

FIGURE 1

Dog. E. Abdominal irradiation with the flaming carbon arc for 60 minutes at 80 cm. every other day for a total of 8 exposures. Heavy blocks denote the period of irradiation.

constant. The relative constancy of this factor has been referred to in the preceding paper.

Dog O was the only animal to show a definite hemoglobin response to irradiation. This animal was given a single massive exposure of 1 hour at 60 cm. (133 g. cal. per sq. cm.). No increased hemoglobin production was observed until the second week following the irradiation. This increase continued during the third week, resulting in the total removal of 23.5 gms. of hemoglobin over and above that removed in the two previous two-week control periods. The long control period during which an average of only 2 gms. per week was removed, and the fact that the increased production did not appear until the second week after the exposure, lessen the possibility of the increase being due to reserve storage factors. It is significant that a second exposure of the same intensity given three weeks after the first was entirely ineffective as were similar exposures of other animals. Whipple and Robscheit-Robbins cite exceptional animals that respond favorably to any diet no matter how ineffective this diet has been shown to be with other animals. It is of interest that along with the increased hemoglobin production, the red cell number and volume remained relatively constant, which is in contrast to their behavior in the other experiments. The response of the reticulocytes, on the other hand, is striking, the average pre-irradiation level being more than doubled 3 days after the exposure, and over four times increased 12 days after. The level was still high at the end of 3 weeks.

To test the possibility that the inability to stimulate hemoglobin formation was due to too severe exposure, four additional experiments were performed in which the animals were given moderate and small doses of carbon arc energy. One animal was irradiated every other day for 1/2 hour at 80 cm. (38.4 g. cal. per sq. cm.) for a total of 7 exposures, while a second animal was irradiated twice on alternate days for 1/2 hour at 80 cm. and four times for 45 minutes at the same distance (57.6 g. cal. per sq. cm.). The third and fourth dogs were irradiated 4 times for 15 minutes at 80 cm. (19.2 g. cal per sq. cm.) at 2 or 3 day intervals, the latter animal being exposed to a second series of similar exposures after a 2-week interval.

The results in all of these experiments were similar to those following massive exposures. The fourth experiment of this series was particularly interesting. This animal had been used as a control for 11 weeks. During this period there was an unusually vigorous hemoglobin production, an average of 9 gms. of hemoglobin being removed each week in order to maintain a constant anemic level. In contrast to this, the average amount of hemoglobin removed weekly over a period of 7 weeks during and after

irradiation was decreased to 6.3 gms., there being no hemoglobin removed during the first week after the first series of exposures and during the irradiation week of the second series.

The above experiments clearly indicate that radiation, at least under these experimental conditions, cannot act as a substitute for dietary deficiencies, a point which has been emphasized in studies in rickets, in which it has been clearly demonstrated that radiation is effective only by virtue of the fact that it enables or permits the organism to utilize more economically materials present but not available. In this connection the experimental procedure used in this work might be criticised as being particularly severe, since it not only removed hemoglobin building stores from the body, but also left the animals only a bare minimum of material which could be effective in the manufacture of hemoglobin. It seemed likely that if some substance, known to have a certain potency in effecting blood regeneration, were added to the maintenance diet, this ability might be enhanced by irradiation of the animal. Whipple and Robscheit-Robbins (1927) showed that dried apricots and peaches, as well as lettuce, are moderately potent stimulants to hemoglobin formation, their addition to the bread diet resulting in the production of from 15 to 45 gms. of hemoglobin per 2-week period over and above the maintenance level. These substances were therefore incorporated in the diet of some of our animals. Dried peaches were added to the bread S diet of two dogs; dried apricots to that of five dogs, and five animals received lettuce in addition to the bread. As a further check, to insure the fact that the particular diet was not responsible for the lack of hemoglobin production, four animals were fed the Cowgill diet (1923) to compare its relative efficiency with that of the Whipple diet in effecting hemoglobin regeneration under these conditions. The procedure was essentially the same as that employed in the previous experiments. The supplemented bread or Cowgill diet was fed for at least two weeks before irradiation was begun and for a similar period after the irradiation was stopped. Various combinations of diet and exposures to the two sources were used on the same or on different animals.

The responses to the irradiation were in all cases similar to those described when bread S alone was fed. With one exception the animals showed an augmented response to the feeding of these substances, the average weekly hemoglobin production being 7 gms. when peaches were added, 15 gms. for apricots and 10 gms. for lettuce. In no case did irradiation, either from the carbon or quartz mercury arc produce any definite increase in hemoglobin production over and above that produced in the control pre-irradiation period. Table I illustrates a typical case. This animal was

fed bread S supplemented by apricots. After a 3-week control period it was given 3 moderate exposures to the carbon arc on alternate days. The amount of hemoglobin removed during this irradiation week was about 2 gms. less than in the previous control period. The amount of hemoglobin removed in the next two weeks (5th and 6th weeks) was about 3 gms. over that removed in a similar control period. The substitution of lettuce for apricots in the diet at this time resulted in an increased hemoglobin production for the following week, which, however, was not maintained in the succeeding irradiation period when the animal was given five moderate exposures to the quartz mercury vapor arc. The amount of hemoglobin formed continued to decrease during the post-irradiation period (10th and 11th weeks).

At this time it was decided to test the relative merits of the Cowgill diet (1923) as compared with the supplemented bread S. A week of feeding of this diet was not accompanied by any increased hemoglobin production. The animal was again irradiated with the quartz mercury vapor arc for the same length of time as previously, and the hemoglobin level remained practically constant, no bleeding (except the amount of blood removed for routine determinations) being necessary to keep it so. At the end of the next two weeks, the hemoglobin level showed definite signs of decreasing, an effect accentuated by a second series of carbon arc exposures. The level began to rise almost immediately when bread S and lettuce were fed. The ineffectiveness of the Cowgill diet in effecting hemoglobin regeneration has been discussed in the preceding paper.

Determinations of white cell number, differentials, red cell fragility, specific gravity and plasma volume were also included in all of these observations. With the exception of the last two functions, no consistent changes were observed which could be ascribed to the irradiation. The number of white cells showed fluctuations considered to be within the normal range. In many cases a relative lymphocytosis was observed during or immediately after the irradiation period, but this was not true in the majority. There was a tendency to increased cell fragility immediately after an exposure in some animals, followed by increased resistance in the post-irradiation period, but this was by no means general. Sharp spurts in the number of platelets were encountered following irradiation in a number of cases where the diet was supplemented, but since similar changes occurred spontaneously in the controls, they are not ascribed to the irradiation. Specific gravity and plasma volume determinations confirmed the results previously published (Mayerson and Laurens, 1928) that irradiation with either the carbon or the quartz mercury vapor arc is

TABLE I.

DOG W—MALE-TERRIER

Weeks	Diet fed	Weight k.	Plasma Volume c.c.	Specific gravity	Red Blood Cells Millions	Reticulocytes p.c. of R.B.C.	R. B. C. hematocrit p.c.	Corpuscular volume cu. μ
1	400 gm. bread S+	7.4	—	1.0454	4.44	—	29.0	65.3
2	200 gms. apricots	—	—	1.0384	4.46	6.3	23.5	52.9
3		7.4	525.0	1.0355	4.67	1.0	20.0	42.8
Irradiation period—Pan Ray Arc—30, 20, 15 minutes at 60 cm. on alternate days.								
4				1.0442	4.87	2.0	28.0	57.4
Post-Irradiation period.								
5		7.6	508.0	1.0415	5.17	2.7	27.0	52.2
6		—	—	1.0387	4.38	2.7	21.0	47.8
7	400 gms. bread S +200 gms. lettuce	7.4	491.0	1.0380	4.51	5.3	21.5	47.6
Irradiation period—Hg arc—5 times—30 min. at 60 cm. on alternate days.								
8		7.2	—	1.0431	4.45	7.0	24.0	53.9
9		7.2	454.0	1.0383	3.98	6.0	19.0	47.7
Post-irradiation period								
10		—	—	1.0474	6.02	10.0	28.0	46.5
11		7.2	523.0	1.0308	4.92	6.0	28.0	56.9
12	115 gms. Cowgill diet	7.2		1.0416	4.30	8.0	19.5	45.3
Irradiation period—Hg arc—5 times—30 min. at 60 cm. on alternate days.								
13		7.0	497.0	1.0407	4.23	5.0	18.0	44.9
14		7.0	—	1.0414	4.26	2.0	21.0	49.4
Post-irradiation period								
15		—	—	1.0391	5.30	3.0	21.0	39.6
16		6.8	543.0	1.0317	3.32	3.0	18.0	54.2
17		—	—	1.0391	3.62	6.0	16.5	45.5
Irradiation period—Pan Ray Arc—30, 20 min. at 60 cm. once each week.								
18		7.0	—	1.0379	3.38	3.0	16.0	47.3
19		—	—	1.0352	2.72	2.0	18.0	66.1
Post-irradiation period								
20	400 gms. bread S	6.8	515.0	1.0354	3.40	4.0	16.0	47.0
21	+200 gms. lettuce	—	—	1.0359	3.92	9.0	17.0	43.0

TABLE I (continued)
DOG W—MALE-TERRIER

Hb level gms. p.c.	Corpuscular Hb gms. $\times 10^{-12}$	P. C. saturation	Fragility*				Platelets $\times 1000$	Whites	Differential					Amounts Hb removed gms.	Total Hb removed gms.
			0.5	0.6	0.7	0.8			S	L	N	E	B		
9.42	20.2	32.6	31	50	47	41	860	10400	23	2	75	0	0	7.86	
7.86	17.6	33.2	41	42	38	35	930	13200	19	3	78	0	0	9.89	
7.04	15.0	34.1	35	33	40	35	2880	10700	23	1	76	0	0	10.45	28.20
8.11	16.6	28.9	35	50	46	44	720	9600	20	14	66	0	0	7.16	7.16
8.30	16.0	30.6	41	49	44	43	1140	11750	22	12	66	0	0	9.33	
7.21	16.4	34.3	36	44	41	35	640	9500	25	2	71	1	0	13.64	
7.40	16.4	34.4	32	35	34	30	620	9750	21	0	79	0	0	19.93	42.90
7.40	16.6	30.7	30	44	43	42	960	6600	17	1	82	0	0	5.18	
6.50	16.3	34.1	34	36	33	33	610	10250	20	1	78	0	1	20.53	25.71
7.95	13.2	28.3	47	49	40	37	800	9700	20	1	79	0	1	9.45	
7.95	16.1	28.2	38	39	40	28	1140	7600	22	0	75	2	1	5.57	
6.50	15.1	33.3	26	33	30	29	480	7450	19	0	70	10	1	7.94	22.96
6.30	14.8	32.9	29	39	34	32	566	8750	15	1	75	8	1	0.0	
6.50	15.2	30.9	32	32	34	30	820	8600	24	0	74	1	1	0.0	
6.85	12.9	32.5	37	32	29	27	400	12500	16	1	81	2	0	0.0	
6.66	20.0	36.8	32	33	29	21	540	10900	18	1	81	0	0	0.0	
5.00	13.8	30.3	27	30	25	24	540	9300	15	1	84	0	0	0.0	
4.80	14.2	30.0	18	28	28	24	400	10400	25	0	73	2	0	0.0	
4.25	15.6	23.5	20	20	23	17	700	7700	15	0	84	1	0	0.0	
3.97	11.6	24.6	27	25	22	18	890	10950	—	—	—	—	—	0.0	
5.35	13.6	31.4	—	—	—	—	830	—	—	—	—	—	—	0.0	

*To Na oxalate hematocrit readings.

accompanied by a diffusion of water from the tissues resulting in increased plasma volume. This change is recovered from after an interval which depends upon the intensity of the exposure.

These observations, contrary to our expectations, decisively indicate that irradiation does not exert any marked influence on hemoglobin regeneration in severe secondary anemia produced by continued bleeding. The various combinations of diet and of exposures which were used, while not absolutely exhaustive, are sufficient to indicate a lack of any outstanding influence, and this is further attested by the fact that the addition of potent foodstuffs at the end of the experiment always evoked a response in these animals. The decreased amounts of hemoglobin formed in many of the animals during and after irradiation is difficult to explain. Determinations of the icteric index in these cases showed no marked increases such as would appear if extensive destruction were occurring, especially so since the decreased formation of hemoglobin persisted for a considerable period after the irradiation had been stopped. The suggestion of McCay (1928), that when an animal is kept anemic for a considerable period the rate of hemoglobin regeneration generally decreases in the second half of any given period, cannot explain these results, since, as previously indicated, these animals show the characteristic responses to the addition of foodstuffs, only moderately effective in increasing hemoglobin production, after periods which unquestionably had exhausted their reserves. The experiments reported in the preceding paper indicated that the strain ensuing upon the feeding of a diet relatively poor in hemoglobin regenerative power stimulated an increased production of small red cells and reticulocytes. The absence of any definite hemoglobin regeneration and the marked responses of these cells in the present experiments suggest that irradiation acts on the mechanism for the production of stroma rather than on that by which hemoglobin is made available.

Summary

Anemia was produced in 24 dogs employing the procedure and diet elaborated by Whipple and Robscheit-Robbins. Carbon and mercury arc irradiation of these animals results in marked and persistent increases in the number of erythrocytes and reticulocytes, the responses being more marked with massive exposures than with smaller doses repeated more often.

An increase in hemoglobin regeneration was noted in only one experiment. In twelve cases the amount of hemoglobin formed during and immediately following the irradiation period is much less than in previous

control periods; in the remaining eleven experiments there was no change in the amount formed. The absence in such radiation of any marked stimulus to hemoglobin formation is evidenced by the fact that peaches, apricots, or lettuce added to the diet of animals which had shown no response to radiation, produce their typical influence. The acceleration of hemoglobin regeneration produced by adding these substances to the diet is also not demonstrably influenced by any dosage of radiant energy as emitted from either of the sources used.

It is a pleasant duty we do in recording here our thanks to Miss Irene M. Sellier for the faithful and painstaking way in which she has aided us in collecting and recording the data which are the basis of these papers.

BIBLIOGRAPHY

Cowgill, G. R., 1923, *Jour. Biol. Chem.*, LVI, 725.
Laurens, H., 1928, *Physiol. Reviews*, VIII, 1.
Laurens, H., and H. S. Mayerson, 1929, *Radiology*, XII, 328.
McCay, C. M., 1928, *Amer. Jour. Physiol.*, LXXXIV, 16.
Mayerson, H. S., 1927, *Proc. Soc. Exp. Biol. Med.*, XXIV, 882.
Mayerson, H. S., and H. Laurens, 1928, *Amer. Jour. Physiol.*, LXXXVI, 1.
Miles, A. L., and H. Laurens, 1926, *Ibid.*, LXXV, 462.
Mitchell, H. S., and L. Schmidt, 1926, *Jour. Biol. Chem.*, LXX, 471.
Osato, S., and S. Tanaka, 1929, *Zeit. f. d. ges. Exp. Med.*, LXV, 692.
Robscheit-Robbins, F. S., 1929, *Physiol. Rev.*, IX, 666.
Robscheit-Robbins, F. S., and G. H. Whipple, 1927, *Amer. Jour. Physiol.*, LXXX, 400.

THE MINERAL, NITROGEN AND FAT CONTENT OF SOME VARIETIES OF MATURE BEAN SEED AND OF STRING BEANS*

By

J. S. McHargue and W. R. Roy

(*From the Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington.*)

Received for Publication—June 9, 1930.

THE purpose of the analyses contained in this paper was primarily to ascertain the copper, manganese, zinc and iron content of some of the more common and widely used varieties of seed and string beans which are important constituents in the diet of man. However, upon making a search of the literature in regard to the mineral matter contained in beans, it was found that only a few varieties have been worked upon and the analyses are usually incomplete.

Samples No. 1 to 10 are the dry, mature seed beans obtained at grocery and seed stores. Sample No. 11 represents the immature seed from green string beans at the time when the seeds were approximately two-thirds grown. Sample No. 12 represents the edible hulls after the beans were removed.

Comments on the results of the analyses

The results show a high and a rather uniform mineral content for the different varieties of beans analyzed. The maximum ash content occurs in samples No. 11 and 12, the seeds and hulls of Kentucky Wonder string beans, the largest percentage being in the hulls. The results for copper, manganese, zinc, and iron in the different varieties analyzed show that they are fairly well supplied with these metals.

Hirano and Mikumo (1) have determined the amount of copper contained in a number of different legumes and most of their results are in agreement with the results for total copper reported in this paper. They also state that from 30 to 40 per cent of the total copper contained in legumes occurs in the seed coats. The senior author (2) has published similar findings in regard to the occurrence of manganese in the seed coats of several kinds of seeds. The calcium and magnesium content are about equal in all the varieties and the quantity is rather large. Beans are well supplied with phosphorus—the maximum content occurs in sample No. 11, the immature

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

TABLE I.

MINERAL, NITROGEN AND FAT CONTENT OF DIFFERENT VARIETIES OF SEED AND STRING BEANS, IN PER CENT OF MOISTURE-FREE MATERIAL

Sample numbers	1	2	3	4	5	6	7	8	9	10	11	12
Varieties	Navy white	Wax black	Pinto brown	Great Northern white	Lima white	Kidney red	Kidney white	Burpee stringless brown	Cut short brown	Ky. wonder brown	Ky. wonder green beans	Ky. wonder edible hulls
Ash, crude	3.34	3.76	4.26	4.29	4.09	3.32	4.01	3.75	4.33	4.14	5.28	7.23
Copper (Cu)	.0011	.0013	.0011	.0015	.0008	.0009	.0010	.0016	.0010	.0011	.0010	.0012
Manganese (Mn)	.0018	.0016	.0014	.0016	.0025	.0015	.0026	.0019	.0014	.0015	.0019	.0039
Zinc (Zn)	.0041	.0026	.0038	.0030	.0046	.0023	.0028	.0037	.0034	.0034	.0046	.0053
Iron (Fe)	.0141	.0156	.0169	.0161	.0150	.0208	.0130	.0190	.0180	.0160	.0270	.0270
Calcium (Ca)	.216	.162	.234	.266	.104	.117	.179	.159	.280	.181	.274	.671
Magnesium (Mg)	.231	.231	.251	.141	.200	.190	.187	.227	.216	.198	.324	.341
Phosphorus (P)	.407	.523	.551	.611	.412	.501	.513	.464	.488	.509	.744	.553
Potassium (K)	1.195	1.27	1.56	1.62	1.89	1.48	1.48	1.41	1.52	1.54	2.00	2.58
Sodium (Na)	.011	.377	.024	.359	.034	.012	.013	.030	.106	.079	.194	.119
Chlorine (Cl)	.034	.028	.037	.035	.034	.046	.023	.041	.052	.062	.056	.051
Sulfur (S)	.275	.316	.225	.348	.227	.318	.275	.266	.355	.273	.867	.191
Fat	2.18	1.60	1.53	1.77	1.09	1.42	1.60	1.40	1.78	1.37	1.41	1.29
Nitrogen (N)	5.27	4.39	4.69	4.62	4.23	4.71	3.54	4.50	3.74	3.84	3.72	2.97
Protein (Nx6.25)	32.95	27.43	29.31	28.88	26.44	29.44	22.13	28.12	23.37	24.01	23.25	18.56

seeds. The results for chlorine represent total chlorine determined by Methods of Analysis, Association of Official Agricultural Chemists, page 43. Sample No. 11 also contains approximately three times as much sulfur as any of the other samples analyzed. The nitrogen content of beans is high and they are one of the principal sources of vegetable protein in the diet of man. However, it has been demonstrated that the proteins of beans cannot be used satisfactorily as a substitute for the proteins of meat, eggs and milk (3). It has been reported that beans are low in cystine and that when this protein is supplied in ample amount the potency of the protein of beans is greatly enhanced. The results for total sulfur in beans show as much or more than is contained in the proteins of animal products. It is also to be observed that the edible hulls contain more mineral matter than the seed. String beans are usually eaten before the seeds have attained maturity. It is apparent that they are rich in mineral matter at this stage of their growth.

Sherman (4) states that in 1908 and 1909, when beriberi was at its worst among the Philippine Scouts, their diet consisted essentially of 12 oz. of beef, 8 oz. of white flour, 8 oz. of potatoes and 20 oz. of rice (ordinarily polished). The change in the ration, as finally decided upon after some months of experimentation, consisted in giving, in place of the 20 oz. of polished rice, 16 oz. of unpolished rice and 1.6 oz. of dried beans. Experiments, performed largely upon fowls, have shown that while beef has some effect in preventing beriberi, an equal weight of beans, peas or peanuts is much more efficacious. Chamberlain states, in fact, that the disease had disappeared as the result of adding the beans to the ration, before the substitution of unpolished for polished rice had been completed.

It is therefore probable that the curative property of beans for beriberi is associated with their relatively high mineral content.

1. Hirano, I., and Mikumo, R., *Jour. Pharm. Soc. Japan*, 1925, No. 525. 992–4; *Chem. Abs.*, 1926, 20, 616.
2. McHargue, J. S., *Jour. Amer. Chem. Soc.*, 1914, XXXVI, 12.
3. McCollum, E. V., Simonds, N., and Pitz, W. J., *Jour. Biol. Chem.*, 1917, XXIX, 521–36.
4. Sherman, H. C., Chemistry of Food and Nutrition, 3rd Ed., 1927, 400.

THE VALIDITY OF FAMILY COEFFICIENTS

By

E. P. CATHCART AND A. M. T. MURRAY

(From the Institute of Physiology, University of Glasgow, Scotland.)

Received for publication—June 9, 1930

THE question of family coefficients is one which, so far as we are aware, has never received the consideration to which it is entitled. Yet it is on this figure that all conclusions about food intake, based on family consumption—the only correct basis for assessment under strictly national conditions—are founded. It is not that no attention has been given to the problem. On the contrary, as reference to the table in Greenwald's (1) paper in *Endocrinology and Metabolism* shows, many attempts have been made to apportion in adequate fashion the food available between the various members of the family studied. When the question is examined more closely it will be found, we believe, that in the majority of these tables the figures are more indicative of an intuitive arrival at a result than the product of direct determination. Admittedly to obtain accurate results from the study of individual households is time-consuming and extremely difficult.

In the course of a recent investigation (2) we had the opportunity of testing the validity of the values commonly employed. Out of a number of families tried, it is to be regretted that, on strict analysis, only five of the results were found to be worthy of credence. These five families totalled in all 22 persons, 10 parents and 12 children. The data were obtained in the course of a study of 154 family diets, each of seven days' duration, by a highly trained investigator. We believe that these results are as nearly exact as such a method can give. We are deeply indebted not only to Miss M. Shanks, our investigator, but to the various house-wives for their patience, care, interest and tolerance.

It may be mentioned here that the man value which we adopted may be regarded as practically a combination of the family coefficients of Atwater and of Lusk. These values are as follows:

Man	1.00	Child 8 to 9–11/12	0.70
Woman	0.83	" 6 " 7–11/12	0.60
Boy 14 up	1.00	" 3 " 5–11/12	0.50
Girl 14 up	0.83	" 2 " 2–11/12	0.40
Child 12 to 13–11/12	0.90	" 1 " 1–11/12	0.30
Child 10 " 11–11/12	0.80	" up " 1	0.20

We had previously adopted this modification as the result of the consideration of repeated actual observations of the food consumed in the course of previous dietetic studies. In order that our results can be recalculated in any value desired, we give a summary of the five families.

TABLE I

	Family	Protein gm.	Fat gm.	Carbohydrate gm.	Calories
A	Man value 3.53	111.06	123.31	382.85	3162
	Father	137.40	166.94	355.25	3564
	Mother	80.45	88.29	292.85	2343
	Boy 14–1/12	92.09	93.57	373.13	2769
	Boy 9	82.09	86.50	330.24	2486
B	Man value 3.03	98.72	102.08	427.76	3097
	Father	126.21	132.01	420.10	3462
	Mother	70.47	70.23	365.69	2433
	Girl 11–6/12	70.72	72.39	355.91	2411
	Girl 2	31.73	34.66	154.40	1079
C	Man value 3.53	91.27	98.28	403.91	2934
	Father	113.93	121.47	436.34	3377
	Mother	72.00	87.23	313.46	2383
	Boy 11–6/12	62.04	69.27	322.28	2211
	Girl 7–11/12	43.41	45.29	235.63	1559
	Boy 1–9/12	30.81	23.68	118.09	828
D	Man value 3.13	84.73	124.37	399.50	3134
	Father	100.06	175.71	398.12	3668
	Mother	66.45	88.63	347.74	2514
	Girl 4–10/12	39.40	48.91	206.90	1460
	Boy 3–4/12	34.42	42.29	197.40	1339
	Girl 1–5/12	24.93	33.75	100.29	827
E	Man value 2.63	92.71	128.02	380.08	3124
	Father	91.26	131.50	396.42	3216
	Mother	70.35	99.62	299.17	2436
	Girl 4	43.30	61.15	163.62	1417
	Boy 1–11/12	38.91	44.42	140.40	1149

The five studies were fortunate, from a comparative point of view, in respect that the daily Calorie value per man of the intake lay between 3162 and 2934. As regards the incomes *per man week* of the five families, they are, in U.S. money, as follows: $3.10, $5.51, $3.42, $4.66 and $3.69. Of this income they spent respectively 88, 53, 54, 60 and 75 per cent on food.

In the first place attention will be devoted to the consumption by the

male wage earner. The following short table shows very clearly that the man in each case obtained a higher calorie intake than he was entitled to from the "man value."

TABLE II

	Calories man value	Calories man consumption	Calories excess	Percentage increase over man value
I	3162	3564	402	12.71
II	3097	3461	364	11.75
III	2934	3377	443	15.09
IV	3133	3667	534	17.04
V	3124	3216	92	2.94

The average of the above analysis shows that in these five families the man obtains in actual fact about 12 per cent more calories than is indicated by the "man value." When the distribution of calories is calculated on the food consumed, it is found that these extra calories were obtained, not from a flat rate increase in all the proximate principles, but from about a 19 per cent increase in protein intake and a 25 per cent increase in the intake of fat. The interpretation is that the wage earner eats the major share of the meat provided in the household. The man in these particular studies consumes about 35 per cent (S.D. 2.8) of the total allowance for the household.

When the food consumption of the mother is examined it is found to be not only well below that of the man but to be definitely below that of the conventional 17 per cent diminution below man value in the family coefficient scale. This is clearly seen from the following table where the percentage diminution below the actual man consumption is calculated as well as the diminution below the family (coefficient) "man value."

TABLE III

	A Calories man value	B Calories man consumption	C Calories mother consumption	Diff. A-C	Percentage diminution	Diff. B–C	Percentage age diminution
I	3162	3564	2343	819	−25.9	1221	−34.2
II	3097	3461	2433	664	−21.4	1028	−29.7
III	2934	3377	2383	551	−18.8	994	−29.4
IV	3133	3667	2514	619	−19.7	1153	−31.4
V	3124	3216	2436	688	−22.0	780	−24.3

These values give average percentage diminution for the woman of more than 21 per cent below "man value" and over 29 per cent below the actual

consumption of the male wage earner. When the values are worked out taking the actual man consumption value as unity for a family coefficient, it would follow, from the data given by these five studies, that the value to be assigned to the adult woman is almost exactly 0.70 instead of the generally accepted 0.83. The five values run as follows: 0.66, 0.70, 0.71, 0.69, and 0.76.

When the nature of the reduction is investigated, it is found that the woman obtains approximately 24 per cent less protein, 24 per cent less fat and 19 per cent less carbohydrate than the "man value" and approximately 36, 39 and 19 per cent less than the actual man consumption.

In the case of the children, unfortunately very few in number, the family coefficient values work out as follows: Boy 14 = 0.78; children 11-12 = 0.70, 0.65; child 9 = 0.70; child 7-8 = 0.46; children 4-5 = 0.40, 0.44; child 3-4 = 0.37; child 2-3 = 0.31; children 1-2 = 0.36, 0.25, 0.23.

TABLE IV

	Average
Man = 1.00	1.00
Woman = 0.52, 0.91, 0.59, 0.73, 0.72, 0.77, 0.54, 0.57, 0.42, 0.54, 0.45, 0.74, 0.57, 0.87, 0.49, 0.84, 0.98, 0.80, 0.68, 0.47	0.66
Boy 14 = 0.92, 0.87. 0.72	0.84
Girl 14 = 0.74, 0.62, 0.61	0.66
Boy 13 = 0.88, 0.60	0.74
Girl 13 = 0.74, 0.98	0.86
Boy 12 = 0.75, 0.76, 0.81, 0.72	0.76
Girl 12 = 0.68, 0.46	0.57
Child 11 = *0.44*, 0.75, 0.89, 0.59	0.67
" 10 = 0.55, 0.62, 0.68	0.62
" 9 = 0.99, 0.55, 0.66	0.73
" 8 = *0.64*, 0.61, 0.61	0.62
" 7 = *0.64*, *0.67*, *0.63*, 0.80, 0.47	0.64
" 6 = *0.68*, *0.71*, *0.47*	0.62
" 5 = *0.52*, 0.73, 0.48, 0.45	0.54
" 4 = *0.41*	0.41

In the above table those values are omitted which were taken from families where Tigerstedt definitely states the father of the family was ill at the time of study.

In the case of the children, the values which refer to female children are italicized.

These figures, few as they are, suffice to arouse the suspicion that the family coefficient values commonly accepted, insofar as they apply to Britain at least, do not possibly represent a true value.

The only comparable series of figures, of which we are aware, is a set collected by Tigerstedt (3). These data, representing 25 families, we have

analysed and as the results detailed below show they, like our own more limited series, raise doubts about the validity of the ordinary standards of family coefficients.

As regards the extent to which the father's consumption exceeded the "man value" figure, calculated on the basis of our coefficients, very few of

TABLE V

Boys Age	No.	Av. wgt. in kilos		Av. Cals. per kilo		Av. total Cals.		Av. total Cals. of Tigerstedt children calc. H&F data	% calc. H&F total Cals. to actual T. values (B/A×100)
		T*.	H&F**	T.	H&F	T. A	H&F B		
14	3	42	42.5	59	80	2442	3400	3360	139.2
13	2	38	38	67	80	2542	3040	3040	119.6
12	7	36	34.2	68	80	2402	2735	2880	113.9
11	3	30.3	31 4	78	80	2359	2510	2424	106.4
10	2	30	29.1	54	80	1917	2330	2400	121.5
9	3	26	26.4	89	80	2277	2110	2080	92.7
8	2	24	24	71	80	1711	1920	1920	112.2
7	2	21.5	21.8	98	80	1953	1745	1720	89.3
6	—	—	—	—	—	—	—	—	—
5	3	17	18.2	104	82	1740	1490	1394	85.6
4	1	15	16.4	104	84	1557	1380	1260	88.6
Girls									
14	4	43.5	45	57	74	2479	3330	3219	134.3
13	2	42	40.6	51	79	2396	3210	3318	133.9
12	2	36	35.8	68	80	2343	2864	2880	122.2
11	1	25	31.5	53	80	1325	2520	2000	190.2
10	1	26	28.5	70	77	1809	2195	2002	121.3
9	—	—	—	—	—	—	—	—	—
8	1	24	23.9	85	76	2030	1815	1824	89.4
7	3	24.3	21.8	76	76	1843	1660	1843	90.1
6	3	21.7	20	85	76	1829	1520	1649	83.1
5	1	19	18.2	73	78	1388	1410	1482	101.6
4	1	14	15.9	81	82	1130	1300	1134	115.1

* Tigerstedt
** Holt & Fales

Tigerstedt's families could be utilized for the calculation as in the majority they had "living in" either adolescent sons in full work or else male servants whose high consumption vitiated the direct assessment. In the nine studies available, there was an average excess consumption above the man value of just over 12 per cent. No stress is laid on the apparent close agreement between this figure and that found in our five studies, as in the pres-

ent series actually only seven of the diets showed an increase; in two there was a slight decrease.

We thought it would be of further interest to compare Tigerstedt's values obtained for his series of children with the arbitrary scale given by Holt and Fales (4). The following table gives the necessary comparative data.

It is astonishing that the American data, as regards standard weights for age, resemble so closely those of Tigerstedt for Finnish children. It would seem, if the Tigerstedt data can be accepted as approximately correct, that Holt and Fales in their deduced scale have overestimated the calorie per kilo body weight needs of the older children and underestimated those of the younger children in the case of the boys whereas with the girls the overestimation is confined in the main to the older children. The differences which exist between the Tigerstedt and Holt and Fales values are clearly discernible in the last column of the table.

The fact that the weights of the Tigerstedt children agree on the whole so well with the standard average figures as given by Holt and Fales compensates to a certain degree for the fewness in numbers and lends a certain value to the differences in observed and calculated calorie intake. It would be, of course, perfectly absurd to draw any far-reaching conclusions from these scanty data, interesting and suggestive though they may be.

Slosse and Waxweiler (5) in their study of the diet of Belgian workmen, published some very scanty data on the family coefficients of four families. It is to be regretted that practically no details are given, especially as to whether the families belonged to the same industrial class as those of the other dietary studies made by them. Their values are high as compared with the direct observations of Tigerstedt and ourselves. They run, taking man as unity, as follows:

Woman (6)	0.93, 0.80, 0.86, 0.94, 0.71, 0.89, 0.84, 0.90, 0.87, 0.81.
Child 9 (2)	0.70, 0.75.
Child 7 (2)	0.60, 0.70.
Child 6 (1)	0.74.

It must be obvious then from the foregoing data, even though they be very limited, that the indiscriminate and uncritical use of family coefficients is frought with danger (See also Greenwood (6)). We do not suggest that the method of family coefficients is useless and should be discarded; such a suggestion would be far from the truth, but we do claim that it is a problem which requires immediate investigation, dispite its difficulty, and laboriousness. The data must be collected from families living under normal con-

ditions of life and labor. Institutional data, no matter how accurately collected, can never fulfil the same end.

REFERENCES

1. Greenwald, Isidor, Endocrinology and Metabolism, New York: 1922, III, 359.
2. Cathcart, E. P., and Murray, A.M.T., A study in Nutrition. Medical Research Council Report, N° 151 H. M. Stationary Office. London.
3. Tigerstedt, Karl, Über die Nährungs Zufuhr des Menschens, Helsingfors, 1915.
4. Holt, L. E., and Fales, H. L., *Amer. Jour. Dis. of Children*, 1921, XXI, 1.
5. Slosse, A., and Waxweiler, E., Enquête sur l'alimentation de 1065 ouvriers belges. Institut. Solvay (Sociologie) Bruxelles, 1910.
6. Greenwood, M., *Metron*, 1924 (Reprint), III, Heft 3-4.

VITAMIN STUDIES XVIII. THE BIOLOGICAL ASSAY OF FOOD MATERIALS FOR VITAMIN A AS INFLUENCED BY YEAST FROM VARIOUS SOURCES*†

By

HANNAH E. HONEYWELL, R. ADAMS DUTCHER, AND JACOB O. ELY

(From the Department of Agricultural and Biological Chemistry, Pennsylvania State College, State College.)

Received for Publication—June 10, 1930.

IN making the biological assay of food materials for vitamin A we have followed, in principle, the curative procedure suggested by Sherman and Munsell (1) which requires a preliminary feeding (depletion) period to rid the tissues of vitamin A, after which sufficient vitamin A-carrying food is added barely to maintain body weight for a period of 8 weeks or to permit of slow growth not to exceed 3 grams per week. Some of our curative experiments, however, have been conducted for 5 instead of 8 weeks for the reason that we have felt that the shorter time was adequate for the information we desired to obtain. We have not found that the additional 3-week period has added much information and the shorter period is less expensive and time consuming.

In the past we have observed, from time to time, considerable variability in the growth response of rats during such experiments and Hume and Smith (2), Sherman and Burtis (3) and others have recorded similar observations.

The experiments described in this paper are the result of an attempt to point out that one of the reasons for divergent results in the assay of vitamin A may lie in the variability of the yeasts or other materials used as a source of the vitamin B complex. This question is not a new one, for Collison, Hume, Smedley-McLean and Smith (4), Hopkins (5) and Luce and Smedley-McLean (6) have already raised questions regarding the variability of yeast. The first-named (4) were of the opinion that their sample of marmite was deficient in vitamin G (B_2), while the others (5, 6) published evidence to show that certain preparations of yeast fat contained vitamin A. Most workers have assumed that yeasts do not contain vitamin A and Hume, Smith and Smedley-McLean (7) have shown that fats from certain

* Read at the XIII International Congress of Physiologists at Boston, Mass., August, 1929.

† Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper No. 504.

yeasts were devoid of this vitamin. Our results tend to show that yeasts may vary in their potency and that this may be due to the presence of vitamin A and to variability in the potency of the vitamin B complex.

In the experiments described in this paper, the rats were taken from our own breeding colony at 21 days of age, at which time they averaged about 40 grams in weight. Litter mates were distributed throughout the experimental groups to eliminate chances for error due to litter peculiarities and at least 10 animals were fed each type of experimental ration. Each growth curve, shown in the charts, represents the average of 10 or more individual growth records. The animals were fed separately in individual cages containing false bottoms made of wire mesh. The ration consisted (in parts per 100) of casein 18, agar 2, salt mixture (McCollum's 185) 4, (8) and dextrin 76. Yeast, in weighed amounts, was fed (daily) separate from the ration and distilled water, containing a trace of iodine, was furnished *ad libitum*. The antirachitic factor was furnished by irradiating the ration (9) with a quartz mercury vapor lamp.

In former years it has been our custom to feed 0.5 gram of yeast daily to furnish the vitamin B complex. Under these conditions we have been able to deplete the vitamin A reserves in 37 days, at which time the vitamin A-carrying food was introduced. The end of the depletion period is determined by a sudden and sharp drop in body weight or by a cessation of growth, or, occasionally, by a bloody nasal discharge or by the appearance of xerophthalmic symptoms. In all the experiments described in this paper, two drops of butter fat (66 mgm.) have been used as a constant and uniform source of vitamin A.

An examination of the records of 700 rats which had been used in various types of vitamin A experiments revealed the fact that not more than 20 per cent of our animals had shown evidence of ophthalmia. During this work we had used a uniform supply of dried yeast as the source of the vitamin B complex. Throughout this paper this yeast will be designated as Yeast I.

Yeast I was received at the laboratory late in 1925 and the supply was sufficient for the ensuing two years. It was with this yeast that we obtained an average growth of about 6 grams per week during the depletion period, which averaged 37 days in length.

In January, 1928, when the supply of Yeast I was nearly exhausted, a second shipment of yeast (Yeast II) was obtained from the same manufacturer. Additional feeding tests (with litter mates of some of the animals described above), identical in all respects except that Yeast II was substituted for Yeast I, gave disturbingly different results. Instead of 6-gram

gains in the depletion period we obtained an average gain of 10 grams for 5 weeks and the average length of the depletion period was extended from 37 to 42 days. The most significant difference, however, was the fact that 90 per cent of our animals were afflicted with definite ophthalmic symptoms as compared with 20 per cent in our previous work. We were confronted, therefore, with an anomalous situation. Our data indicated that Yeast I possessed antiophthalmic properties but poor growth-promoting properties, while Yeast II stimulated growth but possessed no power to prevent the development of the eye disease.

Although the supply of Yeast I was nearly exhausted, we were able to conduct a few experiments in which separate groups of animals received 0.5 gram daily of the two yeasts up to the end of the depletion period. At that point the yeasts were substituted one for the other and the experiment was continued. The ophthalmic symptoms in the group which had previously received Yeast II disappeared upon the introduction of Yeast I but the animals lost weight and died. These experiments led us to believe that we had some evidence to show that vitamin A may consist of two factors. Unfortunately, we were unable to obtain further data on this phase of the problem owing to our inability to obtain an additional supply of Yeast I. It seems evident that either vitamin A consists of two factors or that there is but one factor which has different and separate physiological rôles to play when fed at different levels. We are inclined to the belief that further work will substantiate the hypothesis that vitamin A consists of at least two factors, one which is specific in the prevention and cure of ophthalmia and other epithelial disturbances, and another which possesses the other physiological properties usually ascribed to vitamin A.

Yeast II was then extracted with ether and the ether extract and the yeast residue (now designated as Yeast II(x)) were fed, separately, to different groups of animals. When Yeast II(x) was fed as the source of the vitamin B complex the growth response was practically identical with that obtained with (unextracted) Yeast II except that the depletion period was shortened to 35 days. When the ether extract was freshly prepared and fed at the high level of 0.5 gram daily, growth was stimulated and improvement was observed in animals afflicted with ophthalmia, indicating that a trace of the antiophthalmic factor was present when the extract was fed at this high level, which was roughly 100 times the amount present in the daily allotment of unextracted yeast. At necropsy the animals compared favorably with the control groups which received two drops of butter fat daily. Post mortem examinations of all vitamin A-deficient animals which received Yeast I showed lesions in the lungs and occasionally in the ears.

Since we have been using Yeast III exclusively, the necropsies have revealed pus in the sinuses, both nasal and mastoid, in the throat, kidney, bladder and reproductive system, corroborating the findings of Tyson and Smith and others (10,11,12).

The vitamin A-free control groups died in 10 to 14 days. When this was repeated on Yeast III (produced by another manufacturer) the growth response was surprisingly good but the depletion period was shortened to 32 days, ophthalmia occurred to the extent of 100 per cent, and the animals died in 10 to 14 days following the depletion point, indicating that Yeast III was deficient in vitamin A. A study of the ether extract of Yeast III

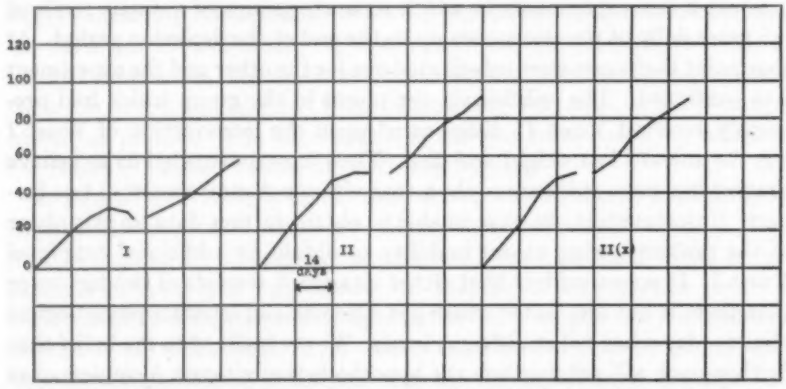

CHART 1.—Average growth curves of the groups which received Yeasts I, II and II(x), showing that the depletion period was lengthened when Yeast II was fed. All animals received 2 drops of butter fat daily during the curative period.

verified these conclusions. When butter fat was added at the end of the depletion period we obtained the greatest growth response we had ever obtained (see Chart 2). It appeared evident, therefore, that the potency of yeast from the standpoint of the vitamin B complex might be the cause of this pronounced growth response.

In order that we might obtain further light on the relation of the vitamin B complex to vitamin A response, four groups of rats were placed on the experimental ration. From the beginning to the end of the experiment each rat received 0.5 gram of yeast, the respective experimental groups being divided according to the type of yeast used. Two drops of butter fat were fed, daily, to each animal at the end of the depletion period. The only experimental variable was the type of yeast, the four groups receiving Yeasts I, II(x), III, and IV respectively. Yeast IV was a dried yeast which had been fortified with an addition of yeast autolysate.

An examination of Graph A, Chart 2, shows that growth response varied with the type of yeast employed. With Yeast I the growth rates during the depletion and curative periods were 6 grams and 6.4 grams, respectively; with Yeast II(x), 10 grams and 8.0 grams, respectively; with Yeast III, approximately 14 grams during both periods; while Yeast IV (from a third manufacturer) produced growth rates of 11 grams and 9 grams, respectively. These data have been corroborated in subsequent experiments. It

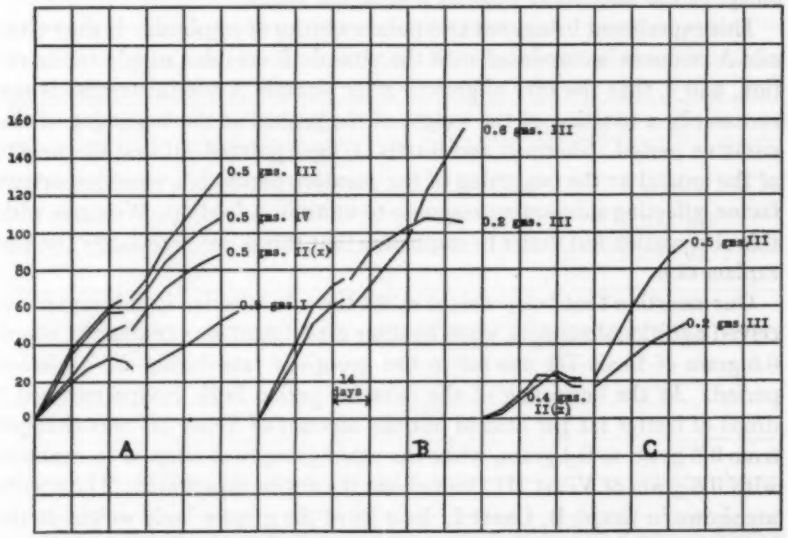

CHART 2.—Graph A shows the average growth curves of rats receiving 0.5 gm. of different yeasts (daily) throughout the experiment and 2 drops of butter fat daily during the curative period.

Graphs B and C show the effect of feeding yeast deficient in the vitamin B complex during the depletion period and changing to a better yeast at the beginning of the curative period. All animals received 2 drops of butter fat (daily) during the curative period.

seems evident, therefore, that the source of the vitamin B complex is a matter of greatest importance in the standardization of vitamin A technique.

Since different brands of yeast influenced the rate of growth when the source of vitamin A was kept constant, we tried the effect of substituting one yeast for another at the end of the depletion period. In Graph C, Chart 2, are shown typical growth curves obtained when two groups of rats received 0.4 gram of Yeast II(x) during the depletion period. At the end of this period both groups were about equal in body weight. At this

point Yeast II(x) was removed and 0.2 gram of Yeast III was substituted in one group while 0.5 gram of Yeast III was added to the diet of the second group. The feeding of two drops of butter fat was started simultaneously in both groups. It will be noted that the growth rate in the curative period was nearly doubled by the increased amount of Yeast III. Since we proved to our own satisfaction that Yeast III was devoid of vitamin A, we are forced to conclude that the increased response was due, in all probability, to the additional vitamin B complex added.

This experiment brings out two points worthy of emphasis: 1, that vitamin A response is correlated with the vitamin B complex supply in the ration, and 2, that the rate of growth after vitamin A administration is not necessarily a function of the weight of the animal at the beginning of the curative period. Sherman and Burtis (3) have pointed out that the weight of the animal at the beginning of the curative period is a most important factor, affecting subsequent response to vitamin A feeding. We agree with this observation but desire to emphasize that this is not necessarily the sole explanation.

Our assertion that body weight is not the sole criterion in this regard received additional support when another experiment was conducted where 0.6 gram of Yeast III was fed to two groups of rats during the depletion period. At the beginning of the curative period both groups received 2 drops of butter fat per animal but the amount of Yeast III was changed from 0.6 gram to 0.2 gram, while the other group was allowed to continue with 0.6 gram of Yeast III throughout the entire experiment. The results are shown in Graph B, Chart 2. In spite of the greater body weight at the beginning of the curative period, the group which received the reduced amount of vitamin B complex in the second period did not grow well after the amount of yeast had been reduced, the growth rate being 6 grams per week as compared with 19 grams per week for the group which received 0.6 gram of yeast throughout the experiment. This was the only experiment conducted in which the animals were all males, which accounts for the greater growth response. In all other experiments males and females were distributed uniformly among the groups.

In the final experiment 11 groups of animals were placed on experiment, of which 7 groups received Yeast III in graduated amounts ranging from 0.2 gram to 0.8 gram by 0.1 gram intervals. Four groups received Yeast II(x) in 0.2 gram intervals, the daily allotments being 0.2, 0.4, 0.6 and 0.8 gram, respectively.

An examination of Graphs A and B, Chart 3, shows that growth responses, in the depletion period and after the introduction of 2 drops of

butter fat per animal, vary with the amount of yeast fed. It is also instructive to note that 0.2 gram of Yeast III produced somewhat better growth than 0.8 gram of Yeast II(x). Here again we have evidence to show that the weight of the animal at the beginning of the curative period is not necessarily an index of the growth response to be expected upon the introduction of vitamin A, for the reason that those animals which received 0.2 gram of Yeast III grew better (11 grams per week) after the introduction

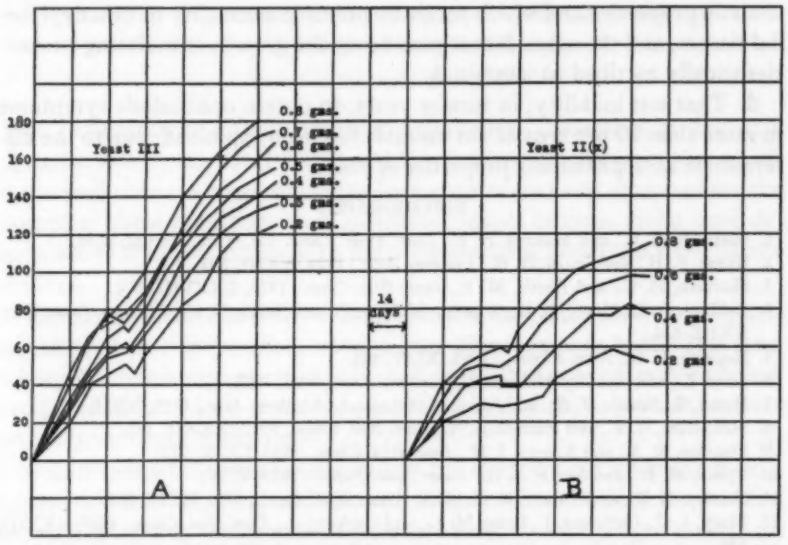

CHART 3.—Graphs A and B show the influence of graduated amounts of yeast on growth in the depletion and curative periods. All animals received 2 drops of butter fat (daily) during the curative period.

of butter fat than those animals which received 0.8 gram of Yeast II(x) and which averaged but 7.5 grams per week. At the beginning of the curative period, however, the latter were somewhat larger than the former.

Experiments are now under way with the hope of ascertaining the rôle played by the B (B_1) and G (B_2) fractions of the vitamin B complex in vitamin A response.

CONCLUSIONS

The results of this work lead us to conclude:—

1. That yeasts made by the same manufacturer may or may not contain traces of vitamin A.

2. That vitamin A response, using the curative method, will vary, depending upon the yeast used as the source of the vitamin B complex.

3. That quantitative results, in assaying foods for vitamin A, will vary, depending upon the potency of the vitamin B complex in the yeast employed.

4. That in the interest of accuracy, laboratory workers can well afford to make periodical tests of their new supplies of yeasts in order to prevent this variable from affecting their vitamin A technique.

5. That vitamin A may consist of two factors, one possessing antiophthalmic properties and which tends to preserve normality of other epithelial tissues, and the other factor possessing the growth-stimulating properties usually ascribed to vitamin A.

6. That our inability, in former years, to obtain ophthalmic symptoms in more than 20 per cent of our animals has been explained, due to the difference in antiophthalmic properties of yeast.

BIBLIOGRAPHY

1. Sherman, H. C., and Munsell, H. E., *Jour. Amer. Chem. Soc.*, 1925, XLVII, 1639.
2. Hume, E. H., and Smith, H. H., *Biochem. Jour.*, 1928, XXIII, 504.
3. Sherman, H. C., and Burtis, M. P., *Jour. Biol. Chem.*, 1928, LXXVIII, 671.
4. Collison, D. L., Hume, E. H., Smedley-McLean, I., and Smith, H. H., *Biochem. Jour.*, 1929, XXIII, 634.
5. Hopkins, F. G., *Jour. Physiol.*, 1912, XLIV, 425.
6. Luce, E., and Smedley-McLean, I., *Biochem. Jour.*, 1925, XIX, 47.
7. Hume, E., Smith, H. H., and Smedley-McLean, I., *Biochem. Jour.*, 1928, XXII, 27.
8. McCollum, E. V., and Simmonds, N., *Jour. Biol. Chem.*, 1917, XXXII, 181.
9. Dutcher, R. A., and Kruger, J. H., *Jour. Biol. Chem.*, 1926, LXIX, 277.
10. Tyson, M. D., and Smith, A. H., *Amer. Jour. Pathol.*, 1929, V, 57.
11. Shurley, B. R., and Turner, R. G., *Jour. Amer. Med. Assoc.*, 1930, XCIV, 539.
12. Macy, I. C., Outhouse, J., Long, M. L., and Graham, A., *Jour. Biol. Chem.*, 1927, LXXIII, 153.

A METABOLISM ROOM FOR THE STUDY OF RATS UNDER CONTROLLED CONDITIONS OF LIGHT AND TEMPERATURE

By

LAURENCE G. WESSON

(From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.)

Received for Publication—June 14, 1930.

IN THE study of certain aspects of metabolism, it seems to us desirable to be able to control the light and temperature conditions under which the animals live. A small room has been constructed and equipped for the control of these conditions over indefinite periods of time, and a brief description of it in connection with the accompanying paper[1] may not be out of place.

The room is an inside room, 600 cubic feet in size, and constructed of hollow tiles. A double-panelled, wooden door gives entrance to it. This door is hinged lengthwise in the middle in such a way that one half may be bolted except when full width of the door is required, and the other half used for ordinary entrance to the room.

A door switch[2] automatically turns on the electric light when the door is opened to enter the room, and turns it off again when the door is opened to leave. The interior of the room is painted black.

In addition to the door and certain cold air ducts which are described later, the room has but one additional opening to the outside,—a small, well-insulated, adjustable shutter that may be used for ventilation. This is usually kept closed since sufficient ventilation is obtained through unavoidable leaks through the wall, around the door, and through the cold air ducts.

An insect-proof rack,[3] suspended from the ceiling, carries 22 individual rat cages.[4] Under these, near the floor, are 2 double "cat" cages[5] in which a

[1] Wesson, L. G., *This Journal*, 1931, III, 503.

[2] Edwards "Eco" door switch, Cat. No. 174, Graybar Electric Co., New York.

[3] Constructed of $\frac{3}{4}''$ cast iron pipe and fittings in the form of shelf supports 1′ x 4′ 6″ x 6′ 6″ high. Four-inch cast iron caps have been tapped and screwed in the 4 uprights in the form of cups near the ceiling. These are filled with liquid petrolatum, as a barrier to insects.

[4] Army Medical School Model, Cat. C. Fig. LC-28, 9″ x 9″ x 15″, Geo. H. Wahmann Co., Baltimore, Md.

[5] Cat. C. Fig. LC-60, 22″ x 20″ x 15″, Geo. H. Wahmann Co., Baltimore, Md.

number of animals may be kept when individual treatment is not deemed necessary.

The temperature of the room is kept approximately constant (28° ± 0.5°C) by the action of two room thermostats.[6] One thermostat, operating a relay[7] and mercury switch when the temperature falls to 27.8°, turns on heating coils[8] and the other, in the same manner, turns on a blower that admits cold air when the temperature rises to 28.2°. The heating coils are placed in a duct through which air is circulated from the bottom to the top of the room at all times by means of an 8″ ventilating fan.[9] Warm air is thus quickly distributed throughout the room. The blower[10] that draws cold air from a refrigerating room nearby is accompanied by a similar blower that returns air from the metabolism room to the refrigerating room and thus the air pressure in both rooms is equalized. The cold air is drawn from a point well within the refrigerating room and upon entering the metabolism room is immediately mixed with part of the constantly circulating air just described.

The adjustment and location of the thermostats are such that the heating coil is used only when the temperature of the outside room is below 27.8°C, while the cold air circuit is drawn upon only when the outside room temperature reaches 28.2°C. Considerable economy is thus effected in the amount of refrigeration required. A recording thermometer[11] placed at an advantageous position in the room is available for use whenever desired.

A definite amount of ultra-violet radiation is provided by means of a mercury arc lamp.[12]

Forced exercise under these standard conditions may be given to the rats by means of a motor-driven exerciser placed under the cage rack. A number of wire baskets held in a frame are slowly revolved 5 times per minute. The rats are thus lightly exercised over long periods of time.

The metabolism room is also used for making calorimetric determinations under standard conditions. An air-stirred water bath that is maintained at 28° ± 0.1°C by an independent temperature control, insures not

[6] Bimetallic room thermostats, Type Q-10 or Type 4010 Minneapolis Honeywell Heat Regulating Co., Minneapolis, Minn.

[7] Wesson, L. G., *Anal. Ed. Jour. Ind. and Eng. Chem.*, 1930, II, 179.

[8] Gilmer Electric Heating Units, Model 810, 110 Volts, 600 Watts, Gilmer Mfg. Corp., Louisa, Va.

[9] Cat. No. 2995, 8″, 110 Volt A.C. Robbins and Myers, Springfield, Ohio.

[10] Sirocco utility sets, No. 1¼″, American Blower Corp., Detroit, Mich.

[11] Serial A46652, 12″,–15 to +40°C, Foxboro Co., Foxboro, Massachusetts (used without the case).

[12] Universal Laboratory, Model, Cat. No. 2640, Hanovia Chemical and Mfg. Co., Newark, N. J.

only that the measurements are made under standard conditions, but eliminates a frequent source of error in calorimetry, such as the temperature variations of parts or of all of the apparatus, during determinations. The closed circuit respiratory apparatus, described in detail elsewhere[1], requires the attention of the operator in the room but a minute or so at the end of each period in which a determination is made.

In addition to the closed circuit apparatus, there is provided a bell jar respiratory apparatus[1] in which an animal may be kept under constant and standard conditions and supplied with a source of outside air in order to collect samples of the expired air for analysis. Just as in the case of the closed circuit apparatus a minute or so only is required to draw off a sample of the air for later analysis, in the adjacent laboratory, with the Henderson-Haldane gas analysis apparatus.

A small sink with hot and cold water faucets allows the rinsing of dishes, and provides a supply of water for the constant temperature bath in the room itself. A water pump attachment has also been found useful for suction.

By providing a room of this character in which not only the variations of temperature and light from day to day and from season to season, but also the important diurnal variations are avoided, it is believed that certain metabolic and vitamin standardizations can be made which would otherwise be impossible, and seasonal variations can be better traced to the real, underlying causes.

THE METABOLIC RATE AND RESPIRATORY QUOTIENTS OF RATS FOLLOWING THE INGESTION OF DEXTRIN AND DURING FASTING*

By

LAURENCE G. WESSON

(From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville.)

Received for Publication—June 14, 1930.

BECAUSE of the increasing use of the white rat in metabolism studies, there has been considerable interest during the past few years in the metabolic rate and respiratory quotients of the rat immediately after feeding, during the basal condition, and during fasting. The length of time required to reach the basal condition is still a matter of uncertainty, as are also the actual basal values. This uncertainty is partly due to the fact that sufficient effort has not been made to carry out these determinations under what might be called standard and uniform conditions. In the work described in the present paper, an attempt has been made to control and standardize the conditions under which the determinations were carried out, and to allow the rat to reach under this environment what is believed to be a more nearly basal condition.

Temperature. It is well known that the metabolic rate of the rat increases as one changes the environmental temperature below or above 28°C. On the basis of this, Goto (1) and Benedict and MacLeod (2) make the statement that the basal rate of the rat should be determined only at 28°. A number of investigators (3, 4, 5, 6,) have selected 27° or 28° as the temperature at which their determinations upon rats were made.

While the metabolic rate changes quickly with variations in the surrounding temperature, a further response is obtained when the subject remains for some time under the influence of the new temperature conditions. A number of investigators (7, 8, 9, 10, 11, 12, 13, 14) have shown that in the tropics or even in the semi-tropics the basal rate of men and women averaged lower and in some cases even 20 per cent less than in temperate climates. von Hoesslin (15) believes that the rate of metabolism of the

* This paper was read in outline form before the American Physiological Society at the Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, March 26-29, 1930.

cells slowly lessens in the hot climate, and that body surface is not the only deciding factor in determining the metabolic rate at a given temperature. Sundstroem (16) gave his rats ample time to adapt themselves to climate environment, but has given no data on the results of his experiments. Hill (17) believed that the metabolism of body cells is raised by an open air life and exposure to cold. Cori and Cori (6) kept their rats at approximately 27° for 24 hours prior to their metabolic rate determinations. Benedict and MacLeod (2) found a higher metabolic rate in rats kept at 21° than in those kept at 28° for 24 hours prior to the determinations which were made at 28°. The seasonal variation in rats found by Benedict and MacLeod (2) may be partially due to differences in the previous environmental temperature.

In the following series of determinations, we have maintained the rats with an environmental temperature of 28° for 3 or more days prior to the beginning of the test period.

Atmospheric humidity and air velocity. These factors are closely allied to that of temperature, as they modify the rate of surface cooling at a given dry bulb temperature. A number of investigators (18, 19, 20) have well shown that the effective temperature and air velocity and not the dry bulb temperature are controlling factors in the rate of metabolism. Their measurements were made on humans, however, in a naked or nearly naked condition where the exposure of the skin would be expected to produce the maximum effect. Clothing, as would be expected, greatly reduces the effect of humidity and air velocity on metabolism. Sundstroem (16) has applied these factors to basal determinations on rats, but has given no figures to show the magnitude of the observed effects when applied to animals well protected by fur. As Richet (21) showed in 1885 by shaving the fur from rabbits, the metabolic rate at a given temperature was enormously increased in the shaved rabbits, while if protected by a cloth covering, these rabbits gave quite normal results. Cluzet (22), working with guinea pigs, found that at 28° there was a slight decrease in the CO_2 output in moisture-saturated air as contrasted with dry air. The effect of normal variations of atmospheric humidity and slight air currents on the metabolic rate of rats at 28° is apparently of secondary importance. We have not attempted to modify the humidity nor to determine the velocity of the mild air currents in the metabolism room used in the following series of determinations.

Barometric pressure. Variations of atmospheric pressure within extremely wide limits are known to have no appreciable effect on the respiratory gas exchange (23 to 40).

Light conditions. Although the rat is a nocturnal animal, in the continuous absence of light and consequently of diurnal variations of light, its activity, as shown by kymographic records, is no greater than under normal conditions. In the following series of measurements we have maintained rats in the practically complete absence of light for 3 or more days prior to the determinations, which were made under the same conditions. This factor has been neglected in previous biocalorimetric work.

TABLE I

SURFACE AREA OF NORMAL RATS AND DEXTRIN REQUIREMENTS PER 24 HOURS

Rat	Surface		Dextrin		Rat	Surface		Dextrin	
Wt.	Area	Increment per gm.	Per 24 hrs.	Increment per gm.	Wt.	Area	Increment per gm.	Per 24 hrs.	Increment per gm.
gm.	cm^2	cm^2	gm.	gm.	gm.	cm^2	cm^2	gm.	gm.
50	131	1.5	2.55	0.030	230	328	0.9	6.39	0.016
60	146	1.4	2.85	0.028	240	337	0.9	6.55	0.016
70	160	1.4	3.13	0.026	250	345	0.8	6.71	0.016
80	174	1.4	3.39	0.025	260	353	0.8	6.87	0.016
90	187	1.3	3.63	0.024	270	361	0.8	7.03	0.015
100	199	1.2	3.87	0.023	280	369	0.8	7.18	0.015
110	211	1.2	4.10	0.022	290	377	0.8	7.33	0.015
120	222	1.1	4.32	0.021	300	385	0.8	7.48	0.015
130	233	1.1	4.53	0.021	310	393	0.8	7.63	0.015
140	244	1.1	4.74	0.020	320	401	0.8	7.78	0.015
150	254	1.0	4.94	0.019	330	408	0.7	7.93	0.014
160	264	1.0	5.13	0.019	340	415	0.7	8.07	0.014
170	274	1.0	5.32	0.019	350	422	0.7	8.21	0.014
180	283	0.9	5.51	0.018	360	429	0.7	8.35	0.014
190	292	0.9	5.69	0.018	370	436	0.7	8.49	0.014
200	301	0.9	5.87	0.017	380	443	0.7	8.63	0.013
210	310	0.9	6.04	0.017	390	450	0.7	8.76	0.013
220	319	0.9	6.21	0.017	400	457	0.7	8.89	0.013

Period of fasting. The usual method of determining the time of the beginning of fasting has been to remove the food cups from the animals without a knowledge of when the animals last ate. Cori and Cori (6), however, in a study of the rate of formation of glycogen, fed glucose by stomach tube and determined the metabolic rate and respiratory quotients for 3 hours after feeding. Árvay (41) fed meat powder and lard and determined the gas exchange for 30 minutes. In the following work we have given the rat a definite amount of dextrin to eat. This was eaten immediately, for the most part, and if a residue remained it was fed to the rat, so that the duration of

the meal was not more than 30 minutes. The periods were then timed from the beginning of this test meal.

Amount and kind of food available for metabolism. Aside from the investigations mentioned in the preceding paragraph, no attempt has been made to control the amount of metabolites available to the animals at the beginning of the fasting period. In this work this was done by a preliminary fast of 18 to 24 hours, followed by a test meal consisting of an amount of dextrin that, based upon an average metabolic rate, would furnish sufficient calories for 12 hours. By the preliminary fasting, available metabolites were reduced to body fat and protein and a minimum of glycogen. At the end of the test meal the rat has an amount of food and food stores that we believe is as definite in amount and kind as can be obtained in a normal animal.

The weight of dextrin for the test meal was calculated on the basis of previous determinations by others of the metabolic rate of the male rat. An average value of 800 Cals. per square meter of body surface per 24 hours, or 0.04 Cal. per square centimeter per 12 hours was assumed. As 1 gm. dextrin by its complete combustion to CO_2 and liquid H_2O furnishes 4.11 Cals. (42), 0.04 Cal. would require 0.009734 gm. dextrin. The weight of dextrin in grams required for the test meal can be found by multiplying by 0.009734 the surface area in square centimeters as calculated from Lee's formula (43 and p. 513). Table I shows the surface area in sq. cm. and the weight of dextrin required for the calories per 24 hours for normal male rats of various body weights on the assumption that all of the 800 Cals. per square meter of skin area are obtained from the dextrin.

Method

In our series the metabolic rate and respiratory quotients were determined for periods up to 44 hours from the beginning of the test meal. These determinations were made at 28° and in the dark in 2 distinct types of apparatus, as follows: 1.—A bell jar in which the rat was placed, and from which the expired air was collected at intervals for analysis in a Henderson-Haldane gas analysis apparatus. 2.—A closed circuit apparatus previously described (44), but now improved in convenience and accuracy. The 20 rats of this series were run repeatedly as follows. Rats A and B were kept in a dark, constant temperature room for 3 or 4 days prior to the fasting and feeding. They were then run at 1-hour periods for the intervals 1 to 10 hours, and 22 to 35 hours, A in the bell jar and B in the closed circuit apparatus. They were then restored to light and the stock diet, and several weeks later were placed in the dark room again and run for the in-

terval 11 to 21 hours, and 34 to 44 hours, in the same apparatus as before. Later the same procedure was repeated, placing B now in the bell jar and A in the closed circuit apparatus. By a similar rotation of the 20 rats it was possible to avoid the effect of the individual variations of the rats on the average of the approximately 500 determinations made.

The rats were male animals, 120 to 220 grams in weight, and maintained, as recommended by Osborne and Mendel (45), on a stock diet of special dog biscuits[1] with greens and milk on alternate days. These determinations were made in September, October and November.

Apparatus

Constant temperature metabolism room (46). A well-insulated inside room of 600 cu. ft. capacity is kept at an approximate temperature of $28 \pm 0.5°C$. Total darkness is obtained at all times except when a person is in the room. At these times the room is illuminated by an incandescent light bulb operated by a door switch. Individual cages in which the rats are kept are supported on an insect-proof rack in this room.

Bell jar apparatus for metabolic rate and respiratory quotient determinations. This apparatus is situated in the constant temperature metabolism room. A bell jar (47) of about 12.5 liters capacity is provided with an inlet tube which reaches to the bottom and an outlet tube which extends from the top, both tubes extending through the rubber stopper of the bell jar and being provided with stopcocks. The bell jar is placed on a glass plate and the ground-glass flange at the bottom is made air-tight by means of petrolatum. Tests for leaks are made before each period by means of a manometer. A small motor manufactured for use in a malted milk mixer was fitted with a short shaft and a fan, and suspended near the top of the inside of the bell jar. The electrical connections for the motor pass through the stopper. The inlet tube to the bell jar is connected to a small, oil-sealed, rotary pump that may be used for pumping air from the outside of the building in and out of the bell jar. A 4-mesh screen in the bell jar $\frac{1}{2}''$ from the glass plate bottom prevents the droppings from being accessible to the rat. The air of the bell jar is sampled by means of gas sampling bulbs and subsequently analyzed in the Henderson-Haldane gas analysis apparatus, with the usual check by duplicate analyses.

To use the apparatus, the animal is placed in the bell jar, and a current of outside air passed through the jar from $\frac{1}{2}$ hour to 1 hour. The stopcocks on the outlet and inlet tubes are then closed, and the expired air is allowed to accumulate for a period which is judged from the size of the rat to be

[1] Manufactured by Potter-Wrightington Co., Inc., Boston.

long enough to give a CO_2 content of 1.5 to 2.5 per cent CO_2. The fan motor is then run for 5 seconds to stir the air, after which an air sample is withdrawn and analyzed in the adjacent laboratory. Blank runs on the apparatus with outside air furnish the CO_2 and O_2 values to be used as a basis for the calculation.

Closed circuit apparatus for metabolic rate and respiratory quotient determinations. This apparatus is also located in the constant temperature

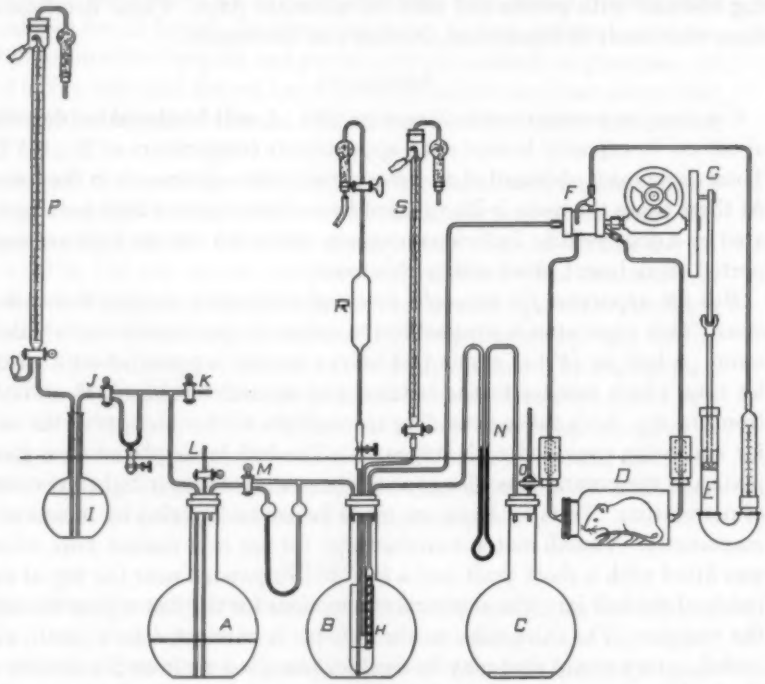

FIGURE 1.

metabolism room. The air in this apparatus is circulated by means of a single-acting, piston pump (E, Figure 1) run by a motor at a speed of about 12 cycles per minute. Liquid petrolatum which fills the cylinder both above and below the piston as far as the U bend of the pump extends, insures that any leak past the piston can be only liquid petrolatum, the meniscus of which may be measured at any time by means of graduations on the glass part of the pump (E). Each down stroke of the pump propels about 80 cc. of air through the 1-liter chamber (D) that contains the ani-

mal, and into a 6-liter flask (B, air storage flask), while each up stroke of the pump sucks about 80 cc. of air from this flask directly into the barrel of the pump without its passing through the animal chamber. The direction that the air takes is determined by the rotary valve (F) attached to the shaft of the crank wheel (G) of the pump.

The CO_2 breathed out by the animal is removed from the air which comes from the animal chamber or jar by means of a spiral scrubber (H) moistened with standardized NaOH solution (approximately half normal). This solution is contained in a test tube, 48 mm. in diameter, supported near the bottom of the flask. The spiral scrubber (H) through which air both enters and leaves the flask is kept moistened with NaOH solution in the following way. When air is drawn from the flask by each up stroke of the pump, a few cubic centimeters of liquid are at first sucked up with it to the top of the scrubber (H) because the end of the scrubber dips a few millimeters below the surface of the solution. When on the down stroke of the pump air is forced into the flask, it passes through the same tube and scrubber by which it left the flask, and thus over a large, freshly moistened surface. In this manner satisfactory absorption of CO_2 is obtained. The amount of CO_2 absorbed in a given period of time is determined at the end of that period by withdrawing exactly 50 cc. out of approximately 55 cc. of the alkali and titrating with standardized HCl in the presence of an excess of $BaCl_2$ solution and phenolphthalein.

A simple form of pump (48) has been successfully adapted to this apparatus and used by several members of the staff of this laboratory. Its pump action depends on the alternate raising and lowering of a mercury reservoir, the direction of the air current being determined by mercury valves. The air, after passing the animal chamber (D), is bubbled into the NaOH solution and passes thence from the top of the air storage flask (B), back to the mercury valve and pump. Since mercury vapor may be harmful in some metabolism work, this form of pump is not recommended in connection with a respiratory calorimeter for general use.

As oxygen is used by the animal, the gas pressure in the system diminishes, as is indicated by a water manometer (N) on the air storage flask. A compensatory flask (C) is connected to the other arm of this manometer. This flask is provided with a stopcock (O) which opens to the air of the room and which is closed at the beginning of the run. The compensatory flask is thus entirely shut off throughout the duration of a run. The temperature and barometric pressure at this time are recorded and are used in the calculations. Moreover, as the compensatory flask contains a small amount of the NaOH solution, such as is used in the air storage flask, the

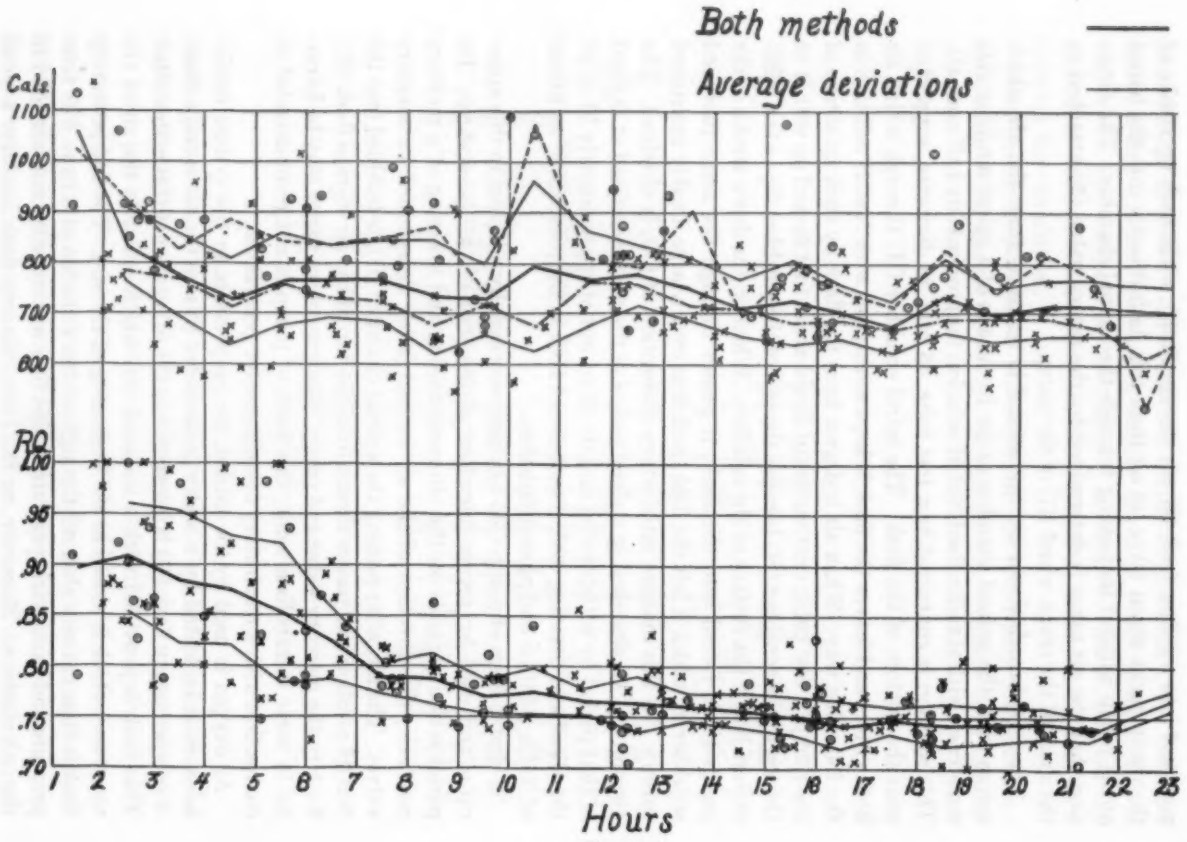

FIGURE 2.

Averages by hours.

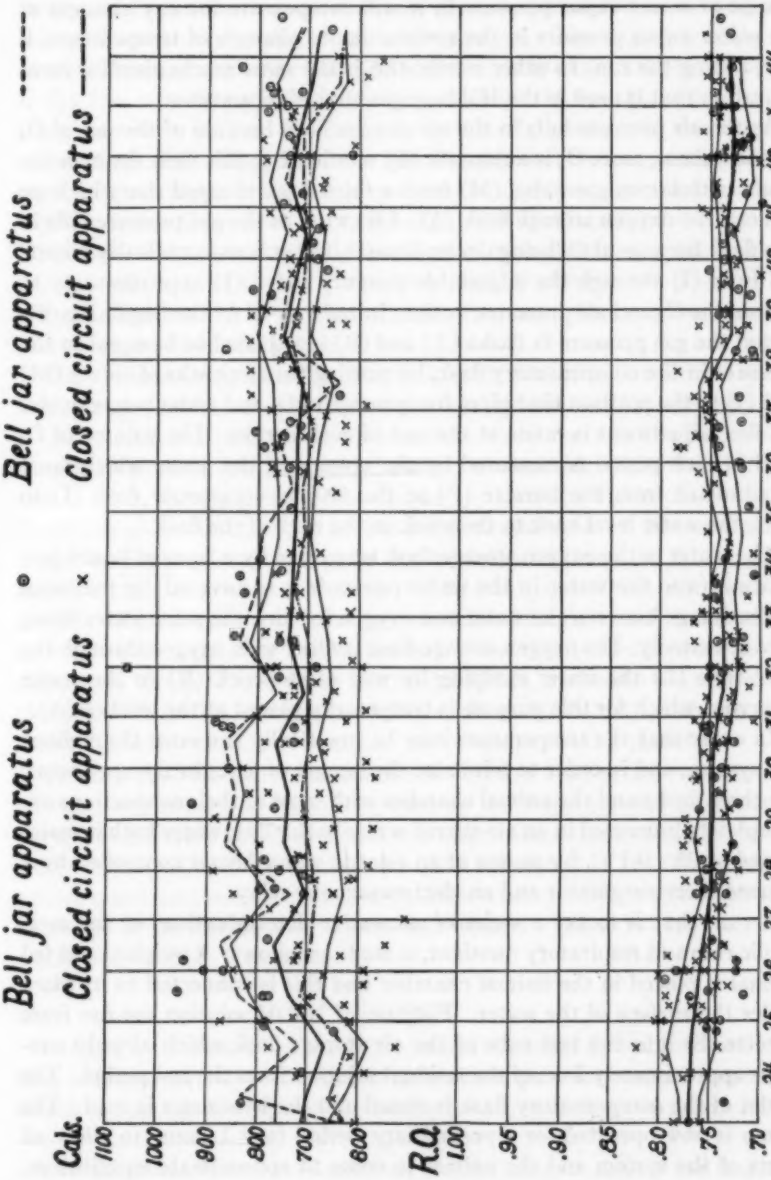

FIGURE 2. (Continued)

change of water vapor pressure in it will compensate for any changes of the water vapor pressure in the system due to changes of temperature, if any, during the run. In other words, this is the same mechanism for compensation that is used in the Haldane gas analysis apparatus.

As the air pressure falls in the air storage flask because of the use of O_2 by the animal, more O_2 is automatically admitted to this flask through the liquid petrolatum gas valve (M) from a third flask of equal size which we will call the oxygen storage flask (A). Likewise, as the gas pressure falls in this flask because of O_2 being drawn from it, water is automatically admitted from (I) through the adjustable mercury valve (J) approximately to restore the O_2 and air pressures to their initial value. At the beginning of a period the gas pressure in flasks (A) and (B) is adjusted to be equal to the pressure in the compensatory flask, by turning the stopcocks of valves (M) and (J) to the position that gives free passage of O_2 and water respectively. Similar adjustment is made at the end of each period. The volume of O_2 used in each period is measured by the volume of the water which must be admitted from the burette (P) to the 500 cc. volumetric flask (I) to bring the water level back to the mark on the neck of the flask.

The water in the oxygen storage flask is covered by a layer of liquid petrolatum (also the water in the water reservoir is so covered) to minimize gas exchange between the water and oxygen (or air), the same water being used repeatedly. The oxygen storage flask is filled with oxygen through the inlet tube (L) the water escaping by way of stopcock (K) to the water reservoir, which for this purpose is temporarily placed at the level of (A).

In order that the temperature may be practically the same throughout the system, and in order to minimize the danger of leaks in the apparatus, the three flasks and the animal chamber with most of their connections are completely immersed in an air-stirred water bath. This water bath is maintained at 28 ± 0.1°C. by means of an electric water heater controlled by a toluene thermoregulator and an electromagnetic relay.

A run, that is to say a series of successive determinations of the metabolic rate and respiratory quotient, is made as follows. A weighed and fed animal is placed in the animal chamber and this is connected to its place under the surface of the water. Fifty cc. of NaOH solution are run from burette (S) into the test tube of the air storage flask which already contains approximately 5 cc. of the residual solution from the last period. The outlet of the compensatory flask is closed and the barometer is read. The pump is now operated for a preliminary period (say 1 hour) to allow all parts of the system and the animal to come to approximate equilibrium. At the end of this preliminary period the pump is stopped at a point in its

stroke that fixes the volume of air in the cylinder of the pump and also leaves the animal chamber open to the system. The stopcock (M) connecting the oxygen storage flask (A) with the air storage flask (B) is opened to the free-running position and water is admitted from the flask (I) until the gas pressure in these two flasks is equal to the pressure in the compensatory flask (C). Fifty cc. of the NaOH solution are then withdrawn by suction into a pipette (R), and transferred to an Erlenmeyer flask containing 50 cc. of a 10 per cent $BaCl_2$ solution. The $BaCl_2$ precipitates the carbonates, and the remaining alkali is titrated with standardized HCl in the presence of phenolphthalein (Winkler's method). In the meantime 50 cc. of fresh alkali are run from burette (S) into the test tube, the pump started, and thus a new period is begun.

The approximately 5 cc. NaOH solution left in the flask at the end of the preliminary period were equivalent to 1/10th of the titration value for the 50 cc. of NaOH solution that were withdrawn. Therefore the sum of the titration values of this 5 cc. and the 50 cc. of the fresh, added NaOH solution is taken as representing the amount of alkali in the test tube at the beginning of the first period. At the end of the first period, the total alkali in the test tube is represented by the titration value of the 50 cc. withdrawn at this time plus 1/10th of this amount, the 1/10th representing the 5 cc. of alkali that remain in the test tube. The difference in the alkali content at the beginning and at the end of the first period represents the alkali used by the CO_2 in terms of standard HCl, and when multiplied by the value of the HCl in terms of CO_2 at 0° and 760 mm., gives the CO_2 under standard conditions breathed out by the rat in this period.[2]

The tables of Boothby and Sandiford (49) are used to convert, by calculation, the volume of O_2 as measured by the water burette to standard dry gas conditions, and to calculate the large calories equivalent to the O_2 used by the rat. These values were then converted to the 24-hour and square meter-surface area basis, using Lee's formula (43), in which the surface area of normal rats in square centimeters $= 12.54 \times$ (body wt.$^{0.60}$), where body weight is in grams.

Results

In Figure 2 are shown the values for the individual determinations (circles, obtained with the Henderson-Haldane apparatus, crosses, with the closed circuit apparatus) and the hourly averages of these and their aver-

[2] The complete respiratory calorimeter or any part thereof may be obtained from George T. Walker & Co., Inc., 324 Fifth Ave. So., Minneapolis, Minn.

age deviations. The hourly standard deviations $\sqrt{\frac{\Sigma d^2}{n-1}}$ and hourly standard deviations from the means $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$ are listed in Table II.

A correction in the metabolic rate values for protein metabolism is unnecessary, as it has been shown that when protein furnishes less than 15 per cent of the total calories, this correction is inappreciable (3, 50).

By the 14th hour the metabolic rate, which at first was high (900 Cals.), reaches a basal value (710 Cals.) that is close to the average of the remaining values. All of the metabolic rate determinations from the 14th to 44th hours inclusive average 718 Cals. per square meter per 24 hours. The average deviation of these values from the mean is 8.4 per cent, the standard deviation is 10.9 per cent, and the standard deviation of the mean is 3.4 per cent. The average of the series obtained in the bell jar apparatus is 771 Cals., in the closed circuit apparatus 695 Cals. The difference between these values (11 per cent or 3 times the standard deviation of the mean) is undoubtedly due to the greater activity of the rat in the bell jar apparatus. The corresponding averages of the respiratory quotients differ by only 1 per cent.

Using Rubner's(51) surface area formula ($S = 9.13W^{\frac{2}{3}}$) in place of Lee's for these basal rate calculations, the average result for all determinations from the 14th to the 44th hours inclusive is 704 Cals. per square meter per 24 hours. The average result for the bell jar apparatus is 764 Cals. and for the closed circuit apparatus, 683 Cals.

Values that have been arrived at by others for the basal rate of the male rat at 28° include the following: Verzár (5), 806 Cals.; Hári (3), 800 Cals.; Goto (1), 825 Cals.; Benedict and MacLeod (2), 800 Cals.; Wu (52), 750 Cals. It is believed that the acclimatization to which the rats of the present series were subjected, before the metabolic rate determinations were made, is responsible for the low basal rate averages obtained (771 and 695 Cals. per square meter per 24 hours).

Although the determinations of the metabolic rate were made on untrained animals, and although it has not been possible to exclude periods of activity of the rats as Benedict's apparatus allows one to do, the magnitude of the deviations compares favorably with that obtained on human subjects from whom absolute relaxation may be obtained, for example, Gephart and Du Bois (57), 5 per cent; Aub and Du Bois (58), 1.1 per cent; Means (56), 7.5 per cent; Benedict, Emmes, Roth and Smith (54), 10 per cent; Benedict (55), 13.9 per cent; normal range of variations, 10 per cent; Benedict and Carpenter (53), standard deviation, 9.3, and standard deviation of the mean, 2.0 per cent (calculated by Krogh (36)).

TABLE II
HOURLY METABOLIC RATE AND RESPIRATORY QUOTIENT AVERAGES

Hours after dextrin	Metabolic rate					Respiratory quotient				
	Average Cals.	S.D.		S.D.of Mean		Average	S.D.		S.D. of Mean	
		Cals.	%	Cals.	%			%		%
1	1072	140	13.1	82	7.6	0.899	0.105	11.7	0.060	6.7
2	833	98	11.7	24	2.8	0.910	0.062	6.9	0.022	2.2
3	777	107	13.8	32	4.1	0.887	0.069	7.8	0.021	2.5
4	728	114	15.7	34	4.7	0.876	0.071	8.1	0.021	2.3
5	768	116	15.1	32	4.2	0.854	0.070	10.2	0.021	2.4
6	764	87	11.4	24	3.1	0.824	0.046	5.5	0.012	1.4
7	765	108	14.1	31	4.0	0.789	0.021	2.6	0.006	0.8
8	732	131	17.8	38	5.2	0.788	0.033	4.2	0.009	1.2
9	728	41	9.9	12	1.7	0.772	0.021	2.7	0.006	0.8
10	793	206	25.9	77	9.7	0.776	0.028	3.6	0.011	1.4
11	770	118	15.1	53	6.7	0.766	0.017	2.2	0.011	1.4
12	775	72	9.2	17	2.1	0.765	0.033	4.3	0.018	1.0
13	750	84	11.0	25	13.3	0.761	0.015	1.9	0.004	0.5
14	710	73	10.1	23	3.1	0.757	0.020	2.6	0.003	0.5
15	723	114	15.7	28	3.8	0.751	0.027	3.5	0.006	0.8
16	704	62	7.8	15	1.8	0.744	0.031	4.2	0.007	0.9
17	674	57	8.4	16	2.3	0.748	0.016	2.1	0.005	0.6
18	736	72	9.7	18	2.4	0.745	0.025	3.3	0.007	0.9
19	694	73	10.5	25	3.6	0.746	0.024	3.2	0.006	0.8
20	712	72	10.1	24	3.3	0.747	0.022	2.9	0.008	1.0
21	712	74	10.3	28	3.9	0.738	0.018	2.4	0.007	0.9
22	582	61	10.5	35	3.3	0.763	0.013	1.7	0.007	0.9
23	699	96	13.7	49	7.0	0.764	0.019	2.5	0.009	1.2
24	661	98	14.8	37	5.6	0.769	0.028	3.6	0.011	1.4
25	736	88	11.9	25	3.3	0.763	0.025	3.2	0.006	0.8
26	752	124	16.5	25	3.3	0.757	0.021	2.7	0.006	0.8
27	701	89	11.0	25	3.1	0.747	0.016	2.1	0.004	0.6
28	741	123	16.4	37	4.9	0.749	0.020	2.6	0.003	0.5
29	736	109	14.8	33	4.4	0.746	0.016	2.1	0.005	0.6
30	762	114	14.9	28	3.6	0.740	0.022	2.9	0.006	0.8
31	704	65	6.6	22	2.7	0.738	0.013	1.7	0.005	0.7
32	742	121	16.3	33	4.4	0.736	0.017	2.3	0.005	0.6
33	730	73	10.0	23	3.1	0.738	0.016	2.1	0.005	0.7
34	724	45	6.2	20	2.7	0.731	0.016	1.7	0.007	1.0
35	720	44	6.0	27	3.6	0.740	0.017	2.2	0.007	0.9
36	719	56	7.7	27	3.7	0.749	0.019	2.5	0.006	0.8
37	710	77	10.5	24	3.3	0.753	0.017	2.2	0.006	0.7
38	744	68	9.1	20	2.6	0.730	0.018	2.4	0.006	0.8
39	723	54	7.4	17	2.3	0.731	0.031	4.1	0.009	1.2
40	706	51	6.4	15	1.8	0.737	0.008	1.0	0.003	0.4
41	736	53	7.2	20	2.7	0.730	0.022	2.9	0.005	0.7
42	720	53	7.2	14	1.9	0.732	0.017	2.3	0.005	0.6
43	696	89	12.7	28	4.0	0.738	0.008	1.0	0.002	0.3
44	671	54	7.9	27	3.8	0.742	0.013	1.7	0.006	0.7

The average hourly respiratory quotient is seen from Figure 2 to have dropped steadily from 0.91 in the 2nd hour after feeding, to 0.744 at the 16th hour. It then rose to 0.769 at the 24th hour, but gradually lessened again to 0.736 at the 32nd hour. Another rise to 0.753 was found at the 37th hour, with a more rapid drop to 0.73 to 0.74 at the 38th and subsequent hours. There is noticed a tendency toward a diurnal variation in the respiratory quotients (higher in the morning, not reflected in the metabolic rate).

The average value for the respiratory quotients from the 14th hour on is 0.743. If the average is taken from the 38th to the 44th hours inclusive, when a more nearly constant value occurs, it is found to be 0.733. The average deviation is 0.0125 or 1.7 per cent; the standard deviation is 0.0159 or 2.1 per cent, and the standard deviation of the mean is 0.00188 or 0.26 per cent. Previous investigators give the following values for fasting rats: Árvay (41), 0.760, 0.750, and 0.737 at 30 hours; Zih (4), 0.743 at 16 hours; Mattill (47), 0.755 at 2 hours; Aszódi (59), 0.78 at 20 hours; Benedict and MacLeod (60), 0.72 to 0.73 at 16 hours; Wu (52), 0.74 at 24 to 29 hours.

The respiratory quotients given above are not corrected for protein metabolism. Cori and Cori (6) have found that rats after fasting for 48 hours metabolize approximately 10 per cent protein. If the respiratory quotient average for the 38th to 44th hours is corrected for 10 per cent protein metabolism, a value of 0.722 is obtained. Cori and Cori (6) give 0.711 for the 48-hour fasting rat.

Summary

By maintaining male rats for some days at the environment under which the respiratory gas exchange is to be determined, the low basal rate of 718 Cals. per square meter per 24 hours was obtained. This basal value was reached in the 14th hour after feeding dextrin and maintained approximately constant for the 30 hours following.

The hourly averages of the respiratory quotients after a dextrin feeding decrease rapidly to 0.744 at the 16th hour thereafter and slowly with several slight diurnal variations to 0.738 at the 43rd hour. The average respiratory quotient for the 38th to 44th hour inclusive is 0.733 uncorrected or 0.722 if corrected for protein metabolism.

The conditions under which these gas exchange studies were carried out are uniform and reproducible as regards temperature, light, previous environment, and reserve of metabolites.

BIBLIOGRAPHY

1. Goto, K., *Biochem. Zeitschr.*, 1923, CXXXV, 107.
2. Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 367.

3. Hári, P., *Biochem. Zeitschr.*, 1924, CLII, 445.
4. Zih, A., *Pflügers Arch. f. d. ges. Physiol.*, 1926, CCXIV, 449.
5. Verzár, F., *Pflügers Arch. f. d. ges. Physiol.*, 1929, CCXXII, 717.
6. Cori, C. F., and G. T. Cori, *Jour. Biol. Chem.*, 1926, LXX, 557.
7. de Almeida, A. O., *Jour. de Physiol. et de Path. gén.*, 1919–20, XVIII, 712, 958.
8. Fleming, W. D., *Jour. Met. Res.*, 1923, IV, 105.
9. Montoro, O., *Compt. rend. 5me Congress de Med., Habana;* cited by E. S. Sundstroem, *Physiol. Rev.*, 1927, VII, 320.
10. Montoro, O., *Revista de Med. y Cirugía de la Habana*, 1927, XXXII, 503; abstracted in *Jour. Amer. Med. Assoc.*, 1927, LXXXIX, 1185.
11. Hafkesbring, R., and P. Borgstrom, *Amer. Jour. Physiol.*, 1926, LXXIX, 221.
12. Hindmarsh, E. M., *Australian Jour. Exp. Biol. and Med. Sci.*, 1927, IV, 225.
13. Sundstroem, E. S., *Univ. Calif. Pub. Physiol.*, 1926, VI; *Physiol. Rev.*, 1927, VII, 320.
14. Steggerda, M., and F. G. Benedict, *Amer. Jour. Physiol.*, 1928, LXXXV, 621.
15. v. Hoesslin, H., *Arch. f. Anat. u. Physiol.*, 1888, 323.
16. Sundstroem, E. S., *Jour. Biol. Chem.*, 1925, LXIII, xli.
17. Hill, L., *Rep. Med. Res. Council*, XXII and 1919, XXXII.
18. Campbell, J. A., D. Hargood-Ash, and L. Hill, *Jour. Physiol.*, 1921, LV, 259.
19. Hill, L., and J. A. Campbell, *Rep. Med. Res. Council*, 1923, LXXIII, 145.
20. McConnell, W. J., C. P. Yagloglou, and W. B. Fulton, *U. S. Pub. Health Rep.*, 1924, XXXIX, 3075.
21. Richet, C., *Arch. de Physiol. normal et path.*, 1885, 3rd series, XVI, 237, 450.
22. Cluzet, J., *Compt. Rend. Acad. Sci.*, 1908, CXLVI, 773.
23. Pflüger, E., *Pflügers Arch. f. d. ges. Physiol.*, 1872, VI, 43.
24. Marcet, W., *Proc. Roy. Soc. London*, 1879, *Series B*, XXVIII, 498.
25. Loewy, A., Untersuchungen über die Respiration und Ciculation bei Aenderung des Druckes, Berlin, 1895.
26. Mosso, U., *Rendiconti della Accad. dei Lincei*, 1896, 221, 273.
27. Schumberg, and N. Zuntz, *Pflügers Arch. f. d. ges. Physiol.*, 1896, LXIII, 461.
28. Loewy, A., J. Loewy, and L. Zuntz, *Pflügers Arch. f. d. ges. Physiol.*, 1897, LXVI, 477.
29. Durig, A., and N. Zuntz, *Arch. f. Anat. u. Physiol.*, 1904, Supplement, 417.
30. Thunberg, T., *Skand. Arch. Physiol.*, 1905, XVII, 133.
31. Zuntz, N., A. Loewy, F. Müller, and W. Caspari, Höhenklima und Bergwanderungen in ihrer Wirkung auf den Menschen, Berlin, 1906.
32. Durig, A., N. Zuntz, and H. v. Schrötter, *Biochem. Zeitschr.*, 1912, XXXIX, 435.
33. Hill, A. V., and A. M. Hill, *Jour. Physiol.*, 1913, XLVI, 81.
34. Douglas, C. G., J. S. Haldane, Y. Henderson, and E. C. Schneider, *Phil. Trans. Roy. Soc. London, Series B*, 1913, CCIII, 185.
35. Hasselbach, K. A., and J. Lindhard, *Biochem. Zeitschr.*, 1915, LXVIII, 265.
36. Krogh, A., Respiratory Exchange of Animals and Man, London, 1916.
37. Barcroft, J., C. A. Binger, A. V. Bock, J. H. Doggart, H. S. Forbes, G. Harrop, J. C. Meakins, and A. C. Redfield, *Phil. Trans. Roy. Soc. London, Series B*, 1923, CCXI, 351.
38. Schneider, E. C., *Amer. Jour. Physiol.*, 1923, LXV, 107.
39. Schneider, E. C., D. Truesdell, and R. W. Clark, *Amer. Jour. Physiol.*, 1924, LXX, 283; 1925, LXXI, 714.
40. Schneider, E. C., and R. W. Clark, *Amer. Jour. Physiol.*, 1925, LXXIV, 334; 1926, LXXV, 297; 1928, LXXXV, 65.
41. Árvay, A., *Biochem. Zeitschr.*, 1928, CXCII, 369.
42. National Research Council, International Critical Tables, New York, 1st ed., 1929, V, 167.
43. Lee, M. O., *Amer. Jour. Physiol.*, 1929, LXXXIX, 24.
44. Wesson, L. G., *Jour. Biol. Chem.*, 1927, LXXIII, 499.
45. Osborne, T. B., and L. B. Mendel, *Jour. Biol. Chem.*, 1926, LXIX, 661.

46. Wesson, L. G., *This Journal*, 1931, III, 499.
47. Mattill, H. A., *Jour. Biol. Chem.*, 1923, LV, 717.
48. Shaw, L. A., A. C. Messer and S. Weiss, *Amer. Jour. Physiol.*, 1929, XC, 107.
49. Boothby, W. M., and I. Sandiford, Laboratory Manual of the Technic of Basal Metabolic Rate Determinations, Philadelphia, 1920.
50. Magnus-Levy, A., in von Noorden's Metabolism and Practical Medicine, Eng. Trans., Chicago, 1907, I, 192.
51. Rubner, M., *Zeitschr. f. Biol.*, 1883, XIX, 536.
52. Wu, H., and T. Chen, *Chinese Jour. Physiol.*, 1929, III, 315.
53. Benedict, F. G., and T. M. Carpenter, *Carnegie Institute Washington Publ.* No. 77, 1907.
54. Benedict, F. G., L. E. Emmes, P. Roth, and H. M. Smith, *Jour. Biol. Chem.*, 1914, XVIII, 139
55. Benedict, F. G., *Jour. Biol. Chem.*, 1915, XX, 263.
56. Means, J. H., *Jour. Biol. Chem.*, 1915, XXI, 263.
57. Gephart, F. C., and E. F. Du Bois, *Arch. Int. Med.*. 1916, XVII, 902.
58. Aub, J. C., and E. F. Du Bois, *Arch. Int. Med.*, 1917, XIX, 840.
59. Aszódi, Z., *Biochem. Zeitschr.*, 1924, CLII, 472.
60. Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, 343.

Editorial Review

THE SPECIFIC DYNAMIC ACTION

IT IS nearly thirty years since Rubner (1) published the notable *Die Gesetze des Energieverbrauchs bei der Ernährung* work, in which he introduced the term *the specific dynamic action* of the foodstuffs. He defined the *requirement of energy* as the amount of energy produced by an animal in 24 hours when it was fasting and free from the thermal influence of cold. He stated that the specific dynamic action was the measure of a specific kind of energy which was evolved after the ingestion of food, this extra energy being the amount over and above the requirement of energy by the organism. He found that the ingestion of meat produced the largest increase of heat in the dog and that the ingestion of carbohydrate produced very little. On the basis of only one experiment he stated that fat occupied an intermediate position which, however, is certainly not the case (2). The specific dynamic action of fat is less than that of carbohydrate, although standard textbooks continue to quote Rubner's figures only.

If what we now call the *basal metabolism* of a typical animal be 100 calories per day and if 100 calories be administered to the animal in each of the several foodstuffs on different days, then the heat production of the animal after receiving meat protein will rise to about 130 calories, after glucose to about 106 calories, and after fat to about 104 calories. These are typical average results.

Rubner's brilliant interpretation of the cause of the phenomena presented by the specific dynamic action of these different foodstuffs deserves to be recalled today, for it shows how a great mind, richly informed by personal experience, yet held in check by due regard for facts, may have true prophetic vision of the truth which those of lesser caliber fail to perceive.

Rubner concluded that energy was liberated in two forms when protein metabolism occurred. One form, which was derived in large measure from the oxidation of sugar, produced from protein in metabolism, was available for the energy requirement of cell life. The remainder was the free heat of thermo-chemical reactions and yielded no power for the maintenance of cell life. Thus in the process of protein metabolism the formation of cleavage products, of acids and of bases, the heat of solution of these, and oxidative changes in the cleavage products themselves, might all have to do with the production of that portion of extra heat which bore no relation to the supply of fuel for the real needs of the cell. The whole matter of the specific dynamic action, therefore, had to do with accessory thermo-chemical reactions of the intermediary metabolism which accompanied the prep-

aration of food fuel for the cells. This energy of the specific dynamic action could be used in replacement of the extra heat developed in the fasting organism under the influence of cold. Thus, although we have seen that, if the metabolism of the living thing be 100 calories at an environmental temperature of 33° and that the ingestion of meat containing 100 calories will cause the metabolism to rise to 130 calories, yet if, through the influence of external cold alone, we increase the metabolism from the basal level of 100 to 130 calories, then on administering the same amount of meat as before the metabolism remains at 130, because the heat of the intermediary thermal reactions may be used in replacement of the heat induced by the influence of cold. The increase in metabolism through the influence of cold is called the *chemical regulation of temperature.*

Concerning the specific dynamic action of the non-nitrogenous foodstuffs, Rubner said that their behavior was analogous to that of protein although less in degree. Thus, starch passed into sugar in the intestines and underwent some loss of energy by fermentation. Perhaps after absorption there was some transformation of carbohydrate into lactic acid which might have a lower biological value as a cell fuel than glucose. There was no evidence whatever that sugar arose from fat, except as suggested by the fact that the reverse process took place, an interpretation which was not permissible in the absence of evidence to establish it. Thus Chauveau (3) had calculated that, if fat were transformed into glucose in metabolism, there would be an energy loss of 34 per cent. The most recent calculation of this sort, made from free energy data by Borsook and Winegarden (4), indicates a loss of 21 per cent of the theoretical maximal energy obtainable. If fat went into sugar its minimal specific dynamic action ought to be, on the basis of considerations developed below, 21 per cent above that of glucose, which is, from the experimental standpoint, demonstrably false.

When one considers the reasoned analysis of Rubner in the light of knowledge obtained during the thirty years since it was uttered, one may well marvel at its accuracy. One has only to recall the work upon the intermediary metabolism of glycogen⇄lactic acid involved in the Hill-Meyerhof theory of muscular contraction to comprehend that in this process the thermo-chemical reactions involved in the production, solution, and neutralization of lactic acid have all been nicely evaluated, and also to recall how Hahn (5) has shown that living muscle may dehydrogenize lactic acid into pyruvic acid with loss of energy. For it is on such reactions as these, in part at least, that we have come to base our interpretations of the specific dynamic action of the foodstuffs.

* * *

In modern work upon the subject the basal metabolism *strictu sensu* is defined as the heat production of a man or animal quietly resting, entirely relaxed, in an environment free from thermal stimuli, and last fed about 18 hours before the metabolism is determined. In some laboratories another word has been added and the determination of the basal metabolic *rate* is in vogue. F. G. Benedict's excellent expression, the *post-absorptive metabolism*, has probably had no popularity because it does not lend itself to abbreviation, as in the case of "doing a basal" or "a rate." For comparative purposes we may consider the basal metabolism as being produced in a test-tube filled with a liquid of definite chemical composition and in a steady state. If into this were projected other chemicals which undergo chemical reactions with the evolution of heat, they would thereby produce specific dynamic action. We may also speak of the basal metabolism in fasting, in diabetes, in typhoid fever, always modifying the term to indicate the variation from the normal, but preserving the requirements of complete rest and freedom from the influence of food ingestion or of cold upon the body.

Richardson and Mason (6) have found that if the basal metabolism of mild or severe diabetic patients be determined, and if these then receive a diet containing the same quantities of protein, fat, and carbohydrate which were actually oxidized, and if this *replacement diet* be subdivided into portions and administered every 2 hours, the group of patients so treated shows an average increase in metabolism of only 1.7 per cent. In one individual the metabolism fell from 830 calories per day without food to 825 calories when a replacement diet containing 834 calories was administered. During the fasting period the respiratory quotient was 0.83; during the period of replacement diet it rose to 0.86 and there were only 5 gm. of sugar in the urine. When fat was added to the replacement diet, so that its heat value was raised from 834 calories to 1254 calories, the metabolism increased slightly to 851 calories, although of 91 gm. of fat ingested only 37 were oxidized. This individual was 34 per cent under his normal weight and his basal metabolism was 39 per cent below the normal for his age and weight. In another individual whose basal metabolism was 1160 calories per day, a replacement diet induced a metabolism of 1220, an increase of 5 per cent, whereas the addition of fat to bring the diet up to 1738 calories produced a metabolism of 1196 calories and an oxidation of only 53 gm. of 130 gm. of fat ingested. Here 77 gm. of ingested fat were deposited in the hungry tissues of this emaciated diabetic individual without there being any concomitant increase in the specific dynamic action. We may conclude from this evidence that, if a mixed diet be so constituted as to conform to

the exact needs of the tissues and be slowly introduced by absorption from the intestines, its specific dynamic action is virtually negligible, and that when there is great undernutrition the gradual digestion, absorption, and deposition of fat, added in excess of the body's requirement of energy, may be accompanied by little or no manifestation of specific dynamic action. This phase of the subject recalls the fact that the digestion, absorption, and deposition of protein is without specific dynamic action, as first shown by Rubner.

One may imagine that in normal life the liver regulates the composition of the blood so as to provide fuel for the maintenance of a constant basal metabolism. If now, the quantity of food which enters the circulation is exactly equal to the quantity needed for metabolism in the tissues, one may understand that such food merely replaces what the liver would have delivered to the blood.

A very different picture from the one outlined above is presented by Mason (7), who determined the effect of a meal of cream and buttered toast on the metabolism of a girl fourteen years old who was 30 per cent under weight and had a basal metabolism 33 per cent under the normal. The meal contained fat, 67 gm.; carbohydrate, 24 gm.; protein, 6 gm.; in all 746 calories, of which 83 per cent were in fat. Under these circumstances the influence of the large influx of fat into the blood stream increased the metabolism from 28.4 calories per square meter body surface per hour to 37.1 calories, or nearly to the level it would have reached had the child been in a normal state of nutrition. The increase in metabolism amounted to 4.9 per cent of the calories present in the fat of the diet during a period of 4 hours, but since the high metabolism was well maintained during the fifth hour, this figure carries no exact valuation. For it has been shown by Murlin and Lusk (2), for the dog at least, that the specific dynamic action of ingested fat continues over a period of 10 or 12 hours, a fact which must not be forgotten when exact determinations are to be made. The figure given, however, has a comparative value, for a few months later, after the body weight of this individual had risen from 29.7 kg. to 42.5 kg. and her basal metabolism from the level of 28.4 to 35 calories per square meter of body surface, then the ingestion of a similar meal containing a slightly larger content of fat raised the metabolism during 4 hours by only 1.8 per cent of the calories ingested, or to a maximum height of 37.8 calories per square meter of surface. Note that after giving fat in quantity to an undernourished individual with a low basal metabolism the heat production per square meter of surface rose to the same level as was found when the subject was restored to nearly normal weight. It appears, therefore, in

under-nutrition, when ample material in the form of fat is introduced from the intestine into the circulation, that the heat production tends to increase to the level it would have attained if the basal metabolism had been at the normal level. In other words, when one speaks of the increased heat production which follows the influx of fat in the blood one may still speak of the metabolism of fat plethora.

* * *

Recent work has abundantly indicated that Lusk's former view that the specific dynamic action of carbohydrate is due to carbohydrate plethora must be modified. A first indication of this appeared in an experiment by Mason (8), who gave 25 gm. of glucose to a man and witnessed a rise in his metabolism from 54 calories per hour to 60 calories within 60 minutes without any change in the R.Q. and with only an insignificant increase in the blood sugar. Deuel (9), working in the Mayo Clinic, gave 75 gm. of glucose to a man and followed the metabolism in subsequent hours. He found that there was no relation between the extra carbohydrate metabolized in the different hours and the specific dynamic action. This was also shown by Schirlitz (9a). Recently Carpenter and Fox (10), with extraordinary exactitude, have followed the course of metabolism in man after giving 5, 10, 15, 20, 52, and 104 gm. of glucose in solution. Five gm. of glucose were just sufficient to cover the carbohydrate oxidized during the basal metabolism period in 1 hour. The metabolism was increased 0.7 calorie during this period, or by 4 per cent of the calories ingested. When 26 gm. of glucose were administered 3.38 gm. were oxidized in the first half hour, an increase of only 0.33 gm. over the basal value, and the metabolism rose from the basal of 30.6 to 32.4 calories per half hour. When in the third half hour the total metabolism had fallen to 31.0 calories, the oxidation of glucose had increased to 4.47 gm. for the period. It is evident from this that the specific dynamic action of carbohydrate cannot be related to the amount of carbohydrate oxidized. In this instance, after giving 26 gm. of glucose the total heat production was increased 3.1 calories, or 3 per cent of the calories ingested. The specific dynamic action was restricted to the first hour and a half, although the increased oxidation of carbohydrate continued for three hours. During the first hour and a half the oxidation of carbohydrate reached 12.2 gm., leaving a residuum of 13.8 gm. in the body the presence of which no longer exerted any specific dynamic action. One may imagine that this carbohydrate was deposited as glycogen in the liver and fed out to the tissues for the support of the basal metabolism in isodynamic replacement of fat.

This suggests the conclusion that the specific dynamic action of glucose is due to the heat of the intermediary reactions between glucose and glycogen.

Increasing the quantities of glucose fed to 52 and 104 gm. increased the height and the duration of the specific dynamic action in human beings. In the hog, after the administration of starch and glucose, the heat production may double, the R.Q. may rise to 1.58, and 56 per cent of the carbohydrate metabolized may be converted into fat. (29) This process of conversion *per se* is accomplished with little loss of energy. But the matter of the high specific dynamic action may be due to the energy reactions of the intermediary metabolites in the glucose⇄glycogen reaction. Whether under these circumstances a true metabolism of plethora is caused by an abundance of metabolites it is impossible to say.

Baur (11) fasted a man several days. On the fourth day the man climbed mountains near the Tegernsee, in the Tyrol. He had no sense of hunger. On the fifth day he rode a bicycle in the Munich stadium for 10 kilometers, then went to the clinic for a rest of three-quarters of an hour. The blood sugar was 58 mg. per 100 cc., but it rose to the high value of 200 mg. after he had received 85 gm. of glucose. The oxygen consumption rose in three successive hours 21, 12, and 10 per cent. Baur wondered at this high metabolism in the light of the fact that the person had been purposely deglycogenated.

The explanation has been given by Dann and Chambers (12), who have fasted dogs for 3 weeks and have then given them 50 gm. of glucose. Ten gm. appeared in the urine (Hofmeister's starvation diabetes), almost none was oxidized in spite of a large increase in blood sugar, and a pronounced specific dynamic action was apparent. These authors drew the conclusion that glucose ingested by fasting animals exerted a specific dynamic action which was not dependent on either the quantity of glucose oxidized or on the concentration of sugar in the blood but was probably due to heat production in the intermediary metabolism, a conclusion also reached by Baur in his series of experiments.

Without any intention of dogmatic assertion, it may be suggested that glucose may not be directly dehydrated and deposited as glycogen, but that it may have to undergo synthesis with phosphoric acid, possibly fragmentation into lactic or pyruvic acid. Hahn (5) has shown that in the anaerobic disappearance of lactic acid from muscle part of it is dehydrogenated and converted into pyruvic acid. Such a reaction involves a considerable loss of energy. Energy again would be required for the reconversion of pyruvic acid into glycogen.

* * *

Much speculation has been indulged in regarding the relative energy values for the performance of a given amount of work when fat and carbohydrate are used for food. Anderson and Lusk (13) found that carbohydrate was 5 per cent more economical than fat. Krogh and Lindhard (14) noted a discrepancy of 11 per cent. Marsh (15) confirmed Krogh but did not find as high a value as his until the eleventh day of the fat diet. The factor of acidosis enters here to restrict the efficiency of the human being when taking a diet free from carbohydrate. Dann and Chambers have found that, if carbohydrate is fed in quantity to a fasting dog, its basal metabolism may fall to a lower level than it had been in fasting. Applying these results to the work of Anderson and Lusk, a recalculation indicates that in the dog at least the energy required to move 1 kilogram of body substance one meter through space demands almost exactly the same isodynamic quantity of fat or of carbohydrate for the unit of work. The energy of the specific dynamic action does not confuse this issue, probably because the metabolites of the incoming glucose may be directly used by the active muscles. The isodynamic replacement of carbohydrate for fat in the production of muscular power would be impossible if fat first underwent conversion into glucose with an energy loss of upward of 20 per cent, as is maintained by the proponents of the theory that sugar alone is the "fuel of life."

It may be added at this point that Murlin and Lusk (2) found that there was arithmetical summation of the specific dynamic action as the result of the combined ingestion of fat and carbohydrate by a resting dog. The R.Q. was of such an order as to indicate that the extra heat production above that produced by carbohydrate alone was due to the oxidation of fat itself. The specific dynamic action of fat and of carbohydrate are therefore dissimilar processes.

* * *

As before stated, the specific dynamic action of protein is far more potent than that of any other foodstuff. A hungry dog, 10 kg. in weight, may devour 1200 gm. of chopped meat in less than 1 minute, and within 3 hours thereafter its heat production may be nearly double its basal metabolism. Chambers and Lusk (16) have shown that when meat is given to the normal dog and then to the same dog when diabetic with phlorhizin, the specific dynamic action appears to be the same. Therefore, the fact that the 58 per cent of glucose formed from protein in metabolism is not oxidized but is eliminated in the urine does not materially influence the specific dynamic action of protein.

Since protein is constructed of individual molecules of amino acids, the specific dynamic action of these separate fragments has been investigated. Glycine and alanine have given potent results; aspartic acid, glutamic acid, and asparagine, negative results. Since asparagine contains the same amount of nitrogen as glycine, it is evident that the specific dynamic action is not proportional to the nitrogen content of the amino acid. Also, since the deamination of asparagine must yield ammonia in the organism, then, if the theorizing of Knoop (17) be true, that amino acids such as glycine and alanine are produced freely in the organism, one would certainly note an increase in the heat production through the intermediary synthesis of these acids and their subsequent metabolism. It appears probable that the well-known synthetic production of glycine after the ingestion of benzoates is only an emergency function.

Chambers and Lusk (16) point out that not only is the specific dynamic action of glycine the same in the normal as in the diabetic dog, in the organism of which it is completely converted into glucose; not only is it twice as great when the dog receives 20 gm. of glycine as when it receives 10 gm., but also that the number of extra calories produced above the level of the basal metabolism (which by definition is the measure of the specific dynamic action) is independent of the size of the dog. Furthermore, whether the animal receives glycine *per os*, subcutaneously, or intravenously, the resultant action is always the same. The specific dynamic action of the material appears to be a constant, resembling the thermo-chemical reactions which might take place when two dissolved substances are brought together in a test-tube.

Rapport and Beard (18) have found that phenyl-alanine has a profound specific dynamic action and that the action of tyrosine is far greater than had hitherto been supposed. The authors have taken the analyses which show the amino acid content of various proteins and have calculated the anticipated increases in heat production which their content of glycine, alanine, leucine, phenyl-alanine, and tyrosine would call for. They find that, after giving meat, the heat production rises 34 per cent, whereas their calculations indicate that the ingestion of the above named amino acids in the quantities present in the meat would have raised it 35 per cent. With gelatin the theoretical increase is 40 per cent and that actually found is 38 per cent. The specific dynamic action of gelatin and meat may therefore be entirely accounted for by the summated effect of five of their component amino acids. Neither casein nor gliadin gave such concordant results. Rapport and Beard (19) believe this to be due to an under valuation of the specific dynamic action of glutamic acid.

The cause of the specific dynamic action of these amino acids has been the motive of much investigation. It is known that beef broth alone (Liebig's extract), in which glycine or alanine is dissolved before their ingestion, causes no increase in the basal metabolism, nor do solutions of urea, of salt, or of materials which would change the acid-base equilibrium, nor does urea formation and elimination which follow asparagine ingestion, nor does glucose ingestion after prolonged fasting and phlorhizin injections, when the sugar is quantitatively eliminated in the urine in this form of accentuated hunger diabetes. Glycine and alanine, both of which substances are completely convertible into glucose in phlorhizin glycosuria, and both of which manifest their usual degree of specific dynamic action under these circumstances, Probably possess this attribute in virtue of the chemical change when the deaminized remainder of the amino acid is converted into glucose.

Unfortunately the pathway of glycine into glucose is unknown, but the probable pathway of alanine may thus be written:

$2C_3H_5NH_2O_2$	$+O_2$	$=2C_3H_4O_2$	$+2NH_3$
2×388 cal.		$=2\times274.5$ cal.	2×91.6 cal. $+44$ cal.
Alanine		Pyruvic acid	Ammonia

$2C_3H_4O_2$	$+2H_2$	$=C_6H_{12}O_6$
2×274.5 cal.	$+132$ cal.	$=681$ cal.
Pyruvic acid		Glycogen hydrate

Two gram molecules of alanine deprived of ammonia have a physiological heat value of (776 cal. − 183 cal.) 593 cal. To convert this into pyruvic acid 44 calories are necessary: to convert two gram molecules of pyruvic acid into glycogen requires 132 calories; in all, therefore, 176 calories are necessary to convert a substance containing 593 calories of physiologically available energy into glucose, or a 30 per cent increase. These calculations are based upon ideas which came after reading the papers of Aubel (20) and of Meyerhof, Lohmann, and Meier (21). Recently Aubel (22) has made other calculations which show that, if alanine passes through pyruvic and lactic acids into glucose and urea, an increase of 38 per cent in the energy physiologically available is required for the reaction. By actual experimentation the specific dynamic action when alanine is given to a dog amounts to about 50 per cent.

Now physical chemists assure us that the calculations given above are antiquated. Adams (23) especially has devoted much thought to this subject. His calculations, by the laws of thermodynamics, show that 1 gm. of

glycine containing 2.10 physiologically available Calories requires a minimum of 0.871 Calorie to drive 1 gm. of glycine into glucose and urea, or 41 per cent of the original caloric content of the substance. In the case of 1 gm. of alanine, containing 3.55 available Calories, 0.337 Calorie is the minimal amount of energy, or 10 per cent of the Calories contained in the original substance. However, the specific dynamic action of glycine is 100 per cent and of alanine 50 per cent of their caloric content when measured upon experimental animals. Adams explains this by stating that his values are minima and, just as external muscular work may require a three to fourfold energy production for its accomplishment, so here this intermediary chemical work may be associated with the production of free heat. Adams also reports that the intermediary conversion into glucose of three of the five carbon atoms contained in glutamic acid may be accomplished without the liberation of free energy, which would account for its lack of specific dynamic action.

Just as the source of muscular energy may be derived from the oxidation of fat in a fasting dog, so in a fasting dog diabetic with phlorhizin the source of energy for the specific dynamic action of protein is obtained from the oxidation of fat. It has been shown in numerous experiments that, after such a phlorhizinized dog has received meat, the non-protein R.Q. is 0.70, or approximately that of fat.

The energy necessary to accomplish the chemical work concerned in the transformation of such substances as glycine and alanine into glucose can not be employed to furnish energy for mechanical work. This principle was established for meat by the work of Rubner (24) and for meat and alanine by that of Anderson and Lusk (13).

A complete analysis of all the factors which enter into the specific dynamic action of protein is quite impossible, but the way of discovery appears open. Recall, for example, the great increase in respiratory activity and heart action after giving meat to a dog.

It may be added that the glands of internal secretion have no direct bearing upon the specific dynamic action. Lusk (25) has shown that, after removal of the thyroid, the specific dynamic action of meat remains unchanged in the dog. Du Bois (26) found that the specific dynamic action of protein and of carbohydrate were within the normal limits in a patient with exophthalmic goiter whose basal metabolism was 75 per cent above the normal, and in a cretin whose basal metabolism was 20 per cent under the normal.

After extirpation of the adrenal glands in dogs, Nord and Deuel (27) found a normal specific dynamic action, and Gaebler (28) has demon-

strated that there is a normal reaction to meat in a hypophysectomized dog. The literature maintaining the contrary viewpoint is cited by these authors.

* * *

When Lusk in 1922 wrote for *Medicine* an article entitled "The Specific Dynamic Action of the Food-Stuffs" he felt that it was his final word on the subject, but within a year a short conversation with Rubner at the International Physiological Congress at Edinburgh in 1923 gave him good reason to doubt the validity of his conclusions. Rubner at that time reaffirmed his belief that the specific dynamic action was due to the heat production of the intermediary metabolism. The evidence accumulated since has tended to justify Rubner's general statement. Perhaps in ten more years this article will appear as faulty as does that of ten years ago. Both Voit and Rubner have recognized a high mortality among theories. If the experimental work be correct, Voit insisted, the important part is achieved. It is sometimes cheering to remember Marat's characterization of Lavoisier, "a charlatan . . . who changes his theories as he does his shoes." G. L.

BIBLIOGRAPHY

1. Rubner, M., Die Gesetze des Energieverbrauchs bei der Ernährung, Leipzig and Vienna, 1902.
2. Murlin, J. R., and Lusk, G., *Jour. Biol. Chem.*, 1915, XXII, 15.
3. Chauveau, A., *Compt. rend. Acad.*, 1897, CXXV, 1070.
4. Borsook, H., and Winegarden, H. M., *Proc. Nat. Acad. Sc.*, 1930, XVI, 559.
5. Hahn, A., *Sitzungsber. Ges. Morph. u. Physiol. in München*, 1929, XXXIX, 24.
6. Richardson, H. B., and Mason, E. H., *Jour. Biol. Chem.*, 1923, LVII, 587.
7. Mason, E. H., *Jour. Clin. Invest.*, 1927, IV, 353.
8. Mason, E. H., *Jour. Clin. Invest.*, 1925–26, II, 521.
9. Deuel, H. J., Jr., *Jour. Biol. Chem.*, 1927, LXXV, 367.
9a. Schirlitz, K., *Biochem. Ztschr.*, 1927, CLXXXIII, 23.
10. Carpenter, T. M., and Fox, E. L., *This Journal*, 1930, II, 389.
11. Baur, H., *Deut. Arch. klin. Med.*, 1929, CLXIV, 202.
12. Dann, M., and Chambers, W. H., *Jour. Biol. Chem.*, 1930, LXXXIX, 675.
13. Anderson, R. J., and Lusk, G., *Jour. Biol. Chem.*, 1917, XXXII, 421.
14. Krogh, A., and Lindhard, J., *Biochem. Jour.*, 1920, XIV, 290.
15. Marsh, M. E., *This Journal*, 1928, I, 57.
16. Chambers, W. H., and Lusk, G., *Jour. Biol. Chem.*, 1930, LXXXV, 611.
17. Knoop, F., *Ztschr. physiol. Chem.*, 1910, LXVII, 489.
18. Rapport, D., and Beard, H. H., *Jour. Biol. Chem.*, 1927, LXXIII, 299.
19. Rapport, D., and Beard, H. H., *Jour. Biol. Chem.*, 1928, LXXX, 413.
20. Aubel, E., *Bull. Soc. Sc. Hyg. Aliment.*, 1924, XII, 257, 416.
21. Meyerhof, O., Lohmann, K., and Meier, R., *Biochem. Ztschr.*, 1925, CLVII, 459.
22. Aubel, E., *Ann. Physiol. et Physico-chim. Biol.*, 1928, IV, 672.
23. Adams, E. T., *Jour. Biol. Chem.*, 1926, LXVII, p. xxi.

24. Rubner, M., *Sitzungsber. d. preuss. Akad. d. Wissensch.*, 1910, XVI, 316.
25. Lusk, G., Proc. XVII. Internat. Congress Med. (London), 1913, Sect. II, 13. Full report by Dann, Chambers, and Lusk in *Jour. Biol. Chem.* to follow shortly.
26. Du Bois, E. F., *Arch. Int. Med.*, 1916, XVII, 915.
27. Nord, F., and Deuel, H. J., Jr., *Jour. Biol. Chem.*, 1928, LXXX, 115.
28. Gaebler, O. H., *Jour. Biol. Chem.*, 1929, LXXXI, 41.
29. Wierzuchowski, M. and Ling, S. M., Journ. Biol. Chem. 1925, LXIV, 697.

INDEX

Absorption and retention of vitamin A in young children, 265.
—, of vitamin A, effect of use of mineral oil upon, 345.
Adrenalin, effect of upon glucose utilization, 204.
Age, in the determination of renal weight, 375.
Alanine, transformation of to pyruvic acid, 527.
Anemia, hemorrhagic, blood studies in, 453.
—, hemorrhagic, effects of radiant energy on, 465.
Apparatus, closed circuit, for metabolic rate and respiratory quotient determinations in rats, 507.
Ascham, Leah, The influence of bulk in the diet upon fecal calcium and phosphorus, 411.
Ashes of rats reared on different diets, 56.
Austin, Elsie M. (See Clough, H. D. and Carman, J. S.), 1.
Auto-oxidation of fats with reference to their destructive effect on vitamin E, 421.

Basal metabolism, influence of high and low protein diet on, in normal women, 79.
— —, recent advances in the study of, 217, 331.
Bean seed and string beans, mineral, nitrogen and fat content of, 479.
Beef muscle in nutrition, preliminary report, 303.
Benedict, Francis G., A multiple-chamber respiration apparatus for rats and other small animals, 161.
— — (see Horst, Kathryn, and Mendel, Lafayette B.), 177.
Biological assay of food materials for vitamin A as influenced by yeast from various sources, 491.
Bischoff, Fritz, and M. Louisa Long, Studies in carbohydrate metabolism following guanidine deglycogenation, 201.
Bladder, urinary, of monkeys on diet deficient in vitamin A, 133.
Blood chemistry, influence of high and low protein diet on, in normal women, 79.
—, studies in hemorrhagic anemia, 453.
Bloomfield, Emily M. (see Rose, Mary Swartz, Vahlteich, Ella McC. and Robb, Elda), 229.

Calcium, body, effect upon of long continued parathyroid administration in rats, 157.
—, content of sixty-day old rats, 158.
—, fecal, influence of bulk in the diet, upon, 411.
Calories, man value of, 485, *et seq.*
Carbohydrate metabolism following guanidine deglycogenation, 201.
—, the utilization of by totally depancreatized dogs receiving no insulin, 99.
Caries, dental, 439.
Carman, J. S. (see Clough, H. D. and Austin, Elsie), 1.
Carr, R. H. (see Gerber, L.), 245.
Casein, diets high in, glycogen formation of rats on, 297.
Cathcart, E. P. and A. M. T. Murray, The validity of family coefficients, 483.
Cereal breakfast foods, nutritive value of, 1, 17.
—, proteins, biological value of, 17.
Childhood, iron requirement in, 229.
Children, metabolism of, 337.
—, study of the absorption and retention of vitamin A, in, 265.
Clayton, Mary M., The comparative value of different food proteins for reproduction and lactation in the rat. II. Milk, egg and meats, 23.
Clough, H. D., J. S. Carman and Elsie M. Austin, The nutritive value of cereal breakfast foods. IV. The response of the human stomach, 1.
Coefficients, family, validity of 483.
Comparative value, of different food proteins for reproduction and lactation in the rat. II. Milk, egg and meats, 23.
Concepcion, Isabelo (see Ocampo, Mariano and Cordero, Narciso), 237.
Cordero, Narciso (see Ocampo, Mariano and Concepcion, Isabelo), 237.
Cummings, Marian J. and H. A. Mattill, The auto-oxidation of fats with reference to their destructive effect on vitamin E, 421.

Day, Paul L., The effect of long continued parathyroid administration upon the body calcium and phosphorus of albino rats, 157.

Deficiencies of synthetic diets in chick nutrition, 39.
Dental diseases, rôle of diet in cause, prevention and cure of, 433.
Determination of the surface area of the white rat, 289.
Dextrin, metabolic rate and respiratory quotient of rats following ingestion of, 503.
Diack, Samuel L., The determination of the surface area of the white rat, 289.
Diet, influence of bulk in, upon fecal calcium and phosphorus, 411.
—, rôle of, in dental diseases, 433.
—, withdrawal of vitamin A from, in the monkey, 121.
Diets, containing approximately 87 per cent of the total caloric value in the form of sucrose, lard and casein, 297.
—, effects of high sugar, on growth and structure of the rat, 61.
—, synthetic, deficiencies of in chick nutrition, 39.
Disease, basal metabolism in, 340.
Dogs, blood studies in hemorrhagic anemia of, 453.
—, Ca and P metabolism in, 418.
—, depancreatized, utilization of carbohydrate by those receiving no insulin, 99.
DuBois, E. F., Recent advances in the study of basal metabolism, Part I, 217.
—, Part II, 331.
Dutcher, R. Adams (see Honeywell, Hannah E. and Ely, Jacob O.), 491.

EDITORIAL review, Recent advances in the study of basal metabolism, Part I, 217.
—,——, Part II, 331.
—,—, The rôle of diet in the cause, prevention and cure of dental diseases, 433.
—,—, The specific dynamic action, 519.
Effect of the use of mineral oil upon the absorption of vitamin A, 345.
Effects of radiant energy on hemorrhagic anemia, 465.
Egg protein, obtained under restricted diets, 245.
— —, value of for reproduction and lactation, 23.
Ely, Jacob O. (see Honeywell, Hannah E. and Dutcher, R. Adams), 491.
Endocrin products, basal metabolism and, 340.
Estrus cycles of rats fed on high sugar diets, 66.
Evans, Herbert M. and Samuel Lepkovsky, On the preparation of a concentrated source of the heat-labile vitamin B, free from contamination with the heat-stable factor G, 353.
Eye conditions of monkeys on diet deficient in vitamin A, 130.

FACTORS which determine renal weight. VII. Protein intake and age. Second paper, 375.
Family coefficients, validity of, 483.
Fasting, metabolic rate and respiratory quotient of rats during, 503.
—, prolonged, metabolism of albino rat during, 177.
Fat content of beans, 479.
—, conversion of, into carbohydrate, 100.
Fats, auto-oxidation of, 421.
Filipinos, basal metabolism of, 237.
Food consumption by different members of family, 484.
—, proteins, comparative value of for reproduction and lactation in the rat, 23.
Further experimental differentiation of vitamins B and G, 395.

GERBER, L. and R. H. Carr, A chemical and immunological study of egg protein obtained under restricted diets, 245.
Gluconeogenesis from fat, 100.
Glucose tolerance of rabbits, 209.
Glycogen formation in rats. II. Diets containing approximately 87 per cent of the total caloric value in the form of sucrose, lard and casein, respectively, 297.
—, liver, of rabbits treated with guanidine compounds, 206.
—, muscle, of rabbits, 207.
Greisheimer, Esther M., and Olga H. Johnson, Glycogen formation in rats. II. Diets containing approximately 87 per cent of the total caloric value in the form of sucrose, lard and casein, respectively, 297.
Growth of monkeys on diet deficient in vitamin A, 126.
—, of Xerophthalmic rats on extracts of urine of children, 269.

——————, on fecal extracts of pre-school children, 272.
Guanidine deglycogenation, studies of carbohydrate metabolism following, 201.
Guinea pigs, sensitization of, to egg proteins, 254.

HANKE, Milton Theo., The role of diet in the cause, prevention and cure of dental diseases, 433.
Hartley, J. G. (see Quinn, E. J. and Whalen, F. B.), 257.
Hawks, Jean E. (see Wang, Chi Che; Huddlestun, Bernice; Wood, Agnes A; and Smith, Edith A.), 79.
Heat production of albino rat during fasting, 196.
——, of various animals referred to surface area, 295.
Hemoglobin, concentration of, in blood of young albino rats, 328.
—, formation, 325.
Hogan, Albert G., and Charles L. Shrewsbury, Deficiencies of synthetic diets in chick nutrition, 39.
Honeywell, Hannah E., R. Adams Dutcher and Jacob O. Ely, Vitamin Studies XVIII. The biological assay of food materials for vitamin A as influenced by yeast from various sources, 491.
Horst, Kathryn, Lafayette B. Mendel and Francis G. Benedict, The metabolism of the albino rat during prolonged fasting at two different environmental temperatures, 177.
Huddlestun, Bernice (see Wang, Chi Che; Hawks, Jean E.; Wood, Agnes A. and Smith, Edith A.), 79.
Hull, F. E. (see McHargue, J. S. and Roy, W. R.), 49.

INFLUENCE of bulk in the diet upon fecal calcium and phosphorus, 411.
—, of high and low protein diet on the basal metabolism and the chemistry of blood and urine in normal women, 79.
Insulin, effect of upon glucose administration, 203.
—, utilization of carbohydrate by totally depancreatized dogs receiving none, 99.
Iron, average daily intake of, by children, 232
——, output of, by children, 233.
—, requirement in early childhood, 229.
Irwin, Margaret H. (see Nelson, P. Mabel and Peet, Louise J.), 303.

JACKSON, C. M., The effects of high sugar diets on the growth and structure of the rat, 61.
Johnson, Olga H. (see Greisheimer, Esther M.), 297.

KERATINIZATION of epithelial structures of monkeys on diet deficient in vitamin A, 136.

LACTATION, comparative value of different food proteins for, 23.
—, some dietary factors influencing, 313.
Lard, diets high in, glycogen formation in rats on, 297.
Laurens, Henry and H. S. Mayerson, The effects of radiant energy on hemorrhagic anemia, 465.
—,—, (see Mayerson, H. S.), 453.
Lepkovsky, Samuel (see Evans, Herbert M.), 353.
Light and temperature control in metabolism room for study of rats, 499.
Liver, nutritional potency of, 49.
—, photographic section of, from rat fed on high sugar diet, 72.
—, weights and glycogen content of rats, 299.
Long, M. Louisa, (see Bischoff, Fritz), 201.
Lusk, Graham, Editorial Review, The specific dynamic action, 519.

MATTILL, H. A. (see Cummings, Marian J.), 421.
——, The nutritive value of the cereal breakfast foods. V. The biological value of some cereal proteins as determined on rats, 17.
MacKay, Eaton M. and Lois Lockard MacKay, Factors which determine renal weight, VII. Protein intake and age. Second paper, 375.
MacKay, Lois Lockard and Eaton M. MacKay, Factors which determine renal weight. VIII. Protein intake and sex, 387.
—,— —, (see MacKay, Eaton M.), 375.
Mayerson, H. S. and Henry Laurens, Blood studies in hemorrhagic anemia, 453.

—, —, (see Laurens, Henry), 465.
McHargue, J. S. and W. R. Roy, The mineral, nitrogen and fat content of some varieties of mature bean seed and of string beans, 479.
—, —, W. R. Roy and F. E. Hull, The nutritional potency of fresh, cooked, dry and alcohol-extracted liver, 49.
Meat in Nutrition, I. Preliminary report on beef muscle, 303.
— — —, II. Some dietary factors influencing lactation, 313.
— — —, III. Hemoglobin formation, 325.
Meats (liver, kidney and beef round) value of for reproduction and lactation, 23.
Mendel, Lafayette B. (see Horst, Kathryn and Benedict, Francis G.), 177.
Mental states, basal metabolism in, 339.
Metabolic rate and respiratory quotients of rats following the ingestion of dextrin and during fasting, 503.
— —, average hourly, of rats, 515.
Metabolism, basal, endocrin products and, 340.
—, —, influence of high and low protein diet on, in normal women, 79.
—, —, in mental states, 339.
—, —, of Filipinos, 237.
—, —, of Orientals, 243.
—, —, of women, 221.
—, —, racial and climatic, 222.
—, Ca and P, in dogs, 418.
—, carbohydrate, following guanidine deglycogenation, 201.
—, in disease, 340.
—, nitrogen, of women on high and low protein diets, 89 *et seq.*
—, of children, 337.
—, of the albino rat during prolonged fasting at two different environmental temperatures, 177.
—, room for the study of rats under controlled conditons of light and temperature, 499.
Milk destruction of vitamins of, during pasteurization, 141.
—, proteins, value of for reproduction and lactation, 23.
Miller, Edgar G. (see Tilden, Evelyn B.), 121.
Mineral, nitrogen and fat content of some varieties of mature bean seed and of string beans, 479.
—, oil, effect of use of upon absorption of vitamin A, 345.
Monkey, response of, to withdrawal of vitamin A from the diet, 121.
Multiple-chamber respiration apparatus for rats and other small animals, 161.
Murray, A. M. T. (see Cathcart, E. P.), 483.

NELSON, P. Mabel, Margaret H. Irwin and Louise J. Peet, Meat in nutrition, I. Preliminary report on beef muscle, 303.
—, —, (see Peet, Louise J. and Smith, Erma A.), 313, 325.
Nickel dissolved from the container during pasteurization, does it catalyze destruction of vitamins in milk, 141.
Nitrogen content of beans, 479.
—, distribution of egg proteins, 249.
Normal standards for basal metabolism, 219.
Nutrition, chick, deficiencies of synthetic diets in, 39.
—, meat in, 303, 313, 325.
Nutritional potency of fresh, cooked, dry and alcohol-extracted liver, 49.
Nutritive value, of cereal breakast foods. IV. The response of the human stomach, 1.
—, V. The biological value of some cereal proteins as determined on rats, 17.

OBESITY, basal metabolism in, 225.
Ocampo, Mariano, Narciso, Cordero and Isabelo, Concepcion. The basal metabolism of the Filipinos, 237.
Orientals, basal metabolism of, 243.
Oxygen consumption of albino rat during fasting, 194.

PARATHYROID administration, effect of on body calcium and phosphorus of albino rats, 157.
Pasteurization of milk, nickel dissolved from the container, during, 141.
Peet, Louise J., Erma A. Smith and P. Mabel Nelson, Meat in Nutrition, III. Hemoglobin formation, 325.
—, —, P. Mabel Nelson and Erma A. Smith, Meat in Nutrition II. Some dietary factors influencing lactation, 313.
—, —, (see Nelson P. Mabel, and Irwin, Margaret H.), 303.
Phosphorus, body, effect upon of long con-

tinued parathyroid administration in rats, 157.
—, content of sixty-day old rats, 158.
—, fecal, influence of bulk in the diet, upon, 411.
Pigeons, egg weights and egg production of, 247.
Pratt, Avery D., Does the nickel dissolved from the container during pasteurization catalyze the destruction of the vitamins of milk, 141.
Preparation of a concentrated source of the heat-labile vitamin B, free from contamination with the heat-stable factor G, 353.
Protein diet, influence of high and low, on basal metabolism and chemistry of blood and urine in normal women, 79.
—, egg, obtained under restricted diets, 245.
—, intake in the determination of renal weight, 375, 387.
Proteins of milk egg and meat, value of for reproduction and lactation, 23.
—, raw, of egg, beef round, liver and kidney, value of for reproduction and lactation, 35.
Pyruvic acid, transformation of alanine to, 527.

QUINN, E. J., F. B. Whalen and J. G. Hartley, The Vitamin B and G contents of certain yeast samples, 257.

RADIANT energy, effects of on hemorrhagic anemia, 465.
Rat, albino, metabolism of during prolonged fasting, 177.
—, growth and structure of, as affected by diets high in sugar, 61.
—, white, determination of surface area in, 289.
Rats, albino, effect of parathyroid upon body Ca and P of, 157.
—, —, hemoglobin concentration in blood of, 328.
—, ashes of, reared on different diets, 56.
—, glycogen formation in, 297.
—, measurements of, fed on high sugar diets, 68.
—, metabolic rate and respiratory quotient of following ingestion of dextrin and during fasting, 503.
—, metabolism room for the study of, 499.
—, multiple chamber respiration apparatus for, 161.
—, sensitization of, to egg proteins, 250.
—, vitamin B and G deficiencies in, 400.
—, xerophthalmic, growth of, on extracts of urine of children, 269.
—, —, —, on fecal extracts of children, 272.
Renal weight, factors which determine, 375, 387.
Reproduction, comparative value of different food proteins for, 23.
—, in rats fed on high sugar diets, 66.
Respiration apparatus, multiple chamber, for rats and other small animals, 161.
Respiratory quotient and metabolic rate of rats following ingestion of dextrin and during fasting, 503.
— —, of fasting rat, 178.
—, quotients, hourly averages of in rats, 515.
Response of rats to prolonged fasting, 199.
—, of the monkey (Macacus Rhesus) to withdrawal of vitamin A from the diet, 121.
Robb, Elda, (see Rose, Mary Swartz, Vahlteich, Ella McC. and Bloomfield, Emily M.), 229.
Rôle, of diet in the cause, prevention and cure of dental diseases, 433.
Rose, Mary Swartz, Ella McC. Vahlteich, Elda Robb and Emily M. Bloomfield, Iron requirements in early childhood, 229.
Rowntree, Jennie I., A study of the absorption and retention of vitamin A in young children, 265.
—, —, The effect of the use of mineral oil upon the absorption of vitamin A, 345.
Roy, W. R. (see McHargue, J. S.), 479.
—, (see McHargue, J. S. and Hull, F. E.), 49.

SANDELS, M. R. (see Sherman, H. C.), 395.
Scurvy in relation to dental disease, 441.
Sex, protein intake and, in the determination of renal weight, 387.
Sherman, H. C. and M. R. Sandels, Further experimental differentiation of vitamins B and G, 395.
Shrewsbury, Charles L. (see Hogan, Albert), 39.
Smith, Erma A. (see Peet, Louise J. and Nelson, P. Mabel), 313, 325.
Smith, Edith A. (see Wang, Chi Che; Hawkes, Jean E.; and Wood, Agnes A.), 79.
Soskin, Samuel, The utilization of carbohy-

drate by totally depancreatized dogs receiving no insulin, 99.
Specific dynamic action, 519.
Stomach, human, evacuation rates of, 12.
Study of the absorption and retention of vitamin A in young children, 265.
Sucrose, diets high in, glycogen formation in rats on, 297.
Sugar, effect of diet high in, on growth and structure of the rat, 61.
Surface area and basal metabolism, 331.
——, determination of, in the white rat, 289.
——, of normal rats, dextrin requirements, 505.

TEETH of rats fed on high sugar diets, 67.
Teeth, pathology of, 438.
—, structure of, 436.
Temperature and light control in metabolism room for the study of rats, 499.
Temperatures, environmental, effect of on metabolism in albino rat, 177.
Tilden, Evelyn B., and Edgar G. Miller, The response of the monkey (Macacus Rhesus) to withdrawal of vitamin A from the diet, 121.

URINE, chemistry of, influence of high and low protein diet on, in normal women, 79.
Utilization, of carbohydrate by totally depancreatized dogs receiving no insulin, 99.

VAHLTEICH, Ella McC. (see Rose, Mary Swartz, Robb, Elda and Bloomfield, Emily M.), 229.
Validity of family coefficients, 483.
Vitamin A, assay of food materials for, 491.
——, content of fecal extracts, 284.
—— of milk, destruction of, during pasteurization, 143.
——, response of monkey to withdrawal of, from the diet, 121.
——, study of the absorption and retention of, in young children, 265.
——, trial of color tests for, 266.
Vitamin B, concentrated source of, free from heat-stable factor G, 353.
—— of milk, destruction of during pasteurization, 148.
——, solubility of, 396.
——, and G contents of certain yeast samples, 257.
—— and G further experimental differentiation of, 395.
Vitamin C deficiency in relation to dental disease, 441 *et seq.*
——, of milk, destruction of, during pasteurization, 150.
Vitamin E, destructive effect of auto-oxidation of fats, upon, 421.
Vitamin G, photographs illustrating deficiencies of, in rats, 403, 405.
——, solubility of, 399.
——, source of vitamin B, free from, 353.
——, (and B) content of certain yeast samples, 257.
—— (and B), further experimental differentiation of, 395.
Vitamins of milk, destruction of by nickel during pasteurization, 141.
Vitamin Studies XVIII. The biological assay of food materials for vitamin A as influenced by yeast from various sources, 491.

WANG Chi Che, Jean E. Hawks, Bernice Huddlestun, Agnes A. Wood and Edith A. Smith, The influence of high and low protein diet on the basal metabolism and the chemistry of blood and urine in normal women, 79.
Wesson, Laurence G., A metabolism room for the study of rats under controlled conditions of light and temperature, 499.
——, The metabolic rate and respiratory quotients of rats following the ingestion of dextrin and during fasting, 503.
Whalen, F. B. (see Quinn, E. J. and Hartley, J. G.), 257.
Wood, Agnes A. (see Wang, Chi Che, Hawks, Jean E. and Smith, Edith A.), 79.

YEAST, amount of necessary for growth in chicks, 41 *et seq.*
—, influence of, on assay of food materials for vitamin A, 491.
—, vitamin B and G content of certain samples, 257.